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Effects of mutations in lymphoid malignancy and immunodeficiency disease

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Department of Laboratory Medicine, Clinical Research Center

Effects of mutations in lymphoid malignancy and immunodeficiency disease

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my wife and family

ABSTRACT

Mutations are responsible for causing various human diseases, including several types of cancer and immunodeficiency syndromes. They can either be involved directly in the pathogenesis of the disease or by influencing the treatment efficacy and cause failure of the patient's response to a specific therapy by adapting the targeted cell to resist the treatment.

In paper I, we generated amino acid substitution variations of BTK at ibrutinib binding site C481, and performed functional analysis for the corresponding proteins. We have also studied various phosphorylations, which are affected by ibrutinib treatment and put our results in a structure-function context. Herein, we demonstrated that substitution of cysteine 481 by threonine (C481T) retained the kinase activity and caused ibrutinib resistance. So we identified a new escape mutant for irreversible BTK inhibitors, which we predict to be found in patients. BTK kinase is completely inactivated by amino acid (codon) replacement of C481 with arginine, phenylalanine, tryptophan or tyrosine, while the activity is severely impaired when C481 replaced by glycine.

In paper II, we have compared the role of N-terminal region domains in the regulation of SYK fusion kinases in terms of phosphorylation, activation, stability and localization. Upon translocation, SYK contributes its kinase domain into two known fusion-proteins, ITK-SYK and TEL-SYK. We have also generated analogous B-cell fusion kinase, BTK-SYK for comparison. The fusion kinases showed differential activation, localization and sensitivity to various inhibitors. Here, we report the activation-mediated nuclear translocation of ITK-SYK, which is rarely seen among kinases. This unique feature of ITK-SYK is therefore of general interest, as its potential relation to ITK-SYK's transforming capability.

In paper III, we have identified a hypomorphic mutation in PGM3 gene replacing isoleucine 322 with threonine in a family with immunodeficient children, described previously. The mutation is severely destabilized and impaired the enzymatic activity of the protein, causing the described phenotype.

LIST OF SCIENTIFIC PAPERS

I. **Abdulrahman Hamasy**, Qing Wang, K. Emelie M. Blomberg, Dara K. Mohammad, Liang Yu, Mauno Vihinen, Anna Berglöf, and C. I. Edvard Smith. Substitution scanning identifies a novel, catalytically active ibrutinib-resistant BTK cysteine 481 to threonine (C481T) variant. *Leukemia* 2017; **31**, 177-185.

II. **Abdulrahman Hamasy**, Alamdar Hussain, Dara K. Mohammad, Qing Wang, Manuela O. Gustafsson, Beston F. Nore, Abdalla J. Mohamed and C. I. Edvard Smith. Differential regulatory effects of N-terminal region in SYK-fusion kinases reveal unique activation-inducible nuclear translocation of ITK-SYK. *Manuscript*

III. Karin E. Lundin, **Abdulrahman Hamasy**, Paul Hoff Backe, Lotte N. Moens, Elin Falk-Sörqvist, Katja B. Elgstøen, Lars Mørkrid, Magnar Bjørås, Carl Granert, Anna-Carin Norlin, Mats Nilsson, Birger Christensson, Stephan Stenmark, C.I. Edvard Smith. Susceptibility to infections, without concomitant hyper-IgE, reported in 1976, is caused by hypomorphic mutation in the phosphoglucomutase 3 (PGM3) gene. Clin Immunol. 2015;161(2):366- 72.

Publications by the author that are not included in the thesis

I. Alamdar Hussain, Dara K. Mohammad, Manuela O. Gustafsson, Merve Uslu, **Abdulrahman Hamasy**, Beston F. Nore, Abdalla J. Mohamed, and C. I. Edvard Smith. Signaling of the ITK (Interleukin 2-inducible T Cell Kinase)-SYK (Spleen Tyrosine Kinase) Fusion Kinase Is Dependent on Adapter SLP-76 and on the Adapter Function of the Kinases SYK and ZAP70. J Biol Chem. 2013 Mar 8;288(10):7338-50.

II. Berglöf A, **Hamasy A**, Meinke S, Palma M, Krstic A, Månsson R, Kimby E, Österborg A, Smith CI. Targets for Ibrutinib Beyond B Cell Malignancies. Scand J Immunol. 2015 Sep;82(3):208-17. Review.

III. Vargas L, **Hamasy A**, Nore BF, Smith CI. Inhibitors of BTK and ITK: state of the new drugs for cancer, autoimmunity and inflammatory diseases. Scand J Immunol. 2013 Aug;78(2):130-9. Review

CONTENTS

LIST OF ABBREVIATIONS

ALL	Acute lymphoblastic leukemia
BCR	B-cell receptor
BLNK	B-cell linker protein
BTK	Bruton's Tyrosine Kinase
CDG	Congenital disorder of glycosylation
CLL	Chronic lymphocytic leukemia
DLBCL	Diffuse large B-cell lymphoma
EBV	Epstein–Barr virus
FL	Follicular lymphoma
ITAM	Immunoreceptor tyrosine based activation motif
ITK	IL-2-inducible T-cell kinase
LAT	Linker for activation of T-cells
MCL	Mantle cell lymphoma
NFAT	Nuclear factor of activated T-cells
NFκB	Nuclear facto kappa B
PGM3	Phosphoglucomutase-3
PH	Pleckstrin homology
PID	Primary immunodeficiency
PI3K	Phosphatidylinositol-3 kinase
PLCγ	Phospholipase Cγ
PTCL	Peripheral T-cell lymphoma
SFK	SRC family kinase
SH2	Src homology 2

SH3	Src homology 3
SLP-76	SH2 domain containing leukocyte protein of 76kDa
SYK	Spleen tyrosine kinase
TCR	T-cell receptor
TEL	Translocation-ETS-Leukemia
TFK	TEC family kinase
TH	Tec homology
WM	Waldenström's macroglobulinemia
XLA	X-linked agammaglobulinemia
Xid	X-linked immunodeficiency
ZAP-70	Zeta-chain associated protein kinase of 70 KDa

1 INTRODUCTION

The human body is made up of trillions of cells with specific functions, and a large number of proteins are involved to control the proper function of the cells. Genes are parts of DNA sequence that regulate the expression and activity of different proteins in various cells/tissues (1,2). Sometimes, an alteration in the single amino acid coding portion of a gene can disturb the synthesis or function of cellular proteins, which in turn will affect on the development and function of the cell and may cause abnormal conditions (3,4).

Mutations can alter the sequence of a gene in the DNA. The defect can occur in single gene or multiple genes, it can also affect part or entire chromosome (5). Mutations divide generally into two major types, somatic mutation and germ line mutation. However, there are several methods for the classification of gene mutations, which based on how the DNA sequence or structure is changed and how this change affects on the function and expression of the corresponding protein.

Mutations play a direct role in the occurrence of variety of human disorders, including several types of cancer and immunodeficiency syndromes. However, the mutation could be embryonically lethal if the affected gene is important for the development. Since the immune system mainly is needed upon birth, immunodeficincies are in general not embryonically lethal. Moreover, mutations can also affect on the patient's response to the treatment and induce drug resistance (6,7). Identification of the genetic alteration contributes to the understanding the molecular mechanism of the disease and facilitates treatment strategies.

1.1 LYMPHOID MALIGNANCIES

Lymphocytes are a subset of white blood cells that circulate in blood and in the lymphatic system. They consist of three basic classes, B-cells, T-cells and natural killer

cells (8,9). Lymphocytes are important part of immune defense that control and mediate various immune responses in both innate and adaptive immunity (10,11).

Wide varieties of hematological malignancies originate from lymphocytes, including more than 40 different types of lymphoid leukemias and lymphomas, each with distinct behavior, prognosis, and pathological characteristics (12,13). Lymphoid malignancies arise from any stages of T and B cells development, and are categorized into five major classes according to WHO classification; Hodgkin lymphoma, precursor lymphoid neoplasm, mature T-cell and natural killer cell neoplasms, mature B-cell neoplasms and immunodeficiency-associated lymphoproliferative disorders.

Like in other malignancies, many types of lymphoid neoplasms have been associated with genetic alterations that promote cancer cell development and survival, such as activating point mutations or chromosomal translocations, and many of these genetic lesions have been identified (14,15). For example, t(7;9)(q34;q34.3) chromosomal translocation that constitutively activating NOTCH1 has been detected in 50% of T-lineage acute lymphoblastic leukemia (T-ALL) (16). The most two common translocation events in B-lineage acute lymphoblastic leukemia (B-ALL) are t(12;21)(p13;q22) translocation that encodes TEL-AML1 leading to activation of JAK-STAT signaling pathway, and BCR-ABL1 translocation event that present in 25% of adult ALL (17). Moreover, t(5;9) (q33;q22) chromosomal translocation generating ITK-SYK chimeric oncogene recurrently detected in a subset of peripheral T-cell lymphoma (PTCL) (18).

Furthermore, gain-of-function genetic alterations affecting CARD11 and CD79B have been detected in diffuse large B-cell lymphoma (DLBCL), in addition to the mutations of the adaptor protein MYD88 that recognized in Waldenström's macroglobulinemia (WM) and DLBCL, which support the survival of malignant cells (19,20). Sometime these genetic alterations do not cause the disease, rather they induce drug resistance, such as C481S variation in Bruton's Tyrosine Kinase (BTK), that induces ibrutinib resistance in a subset of patients with chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) (21).

1.2 T-LYMPHOCYTE

T-lymphocytes are essential component of the immune system and play a pivotal role in cell-mediated immune responses. They originate in bone marrow from common lymphoid progenitors; afterward the immature T-cells migrate into thymus in which they undergo several developmental stages characterized by co-receptor expression. The double negative $CD4^{-}CD8^{-}$ thymocytes start the maturation and progress to become double positive $CD4^{+}CD8^{+}$ thymocytes that undergo negative and positive selection processes and transform into mature single positive $CD4^{+}$ or $CD8^{+}$ T-cells with αβ-T-cell receptor (TCR) expression (22-25). Thereafter, the naïve T-cells are ultimately released in peripheral lymphoid tissues (26).

T-cells are involved in various immunological functions and divided mainly into three distinct lineages: 1) helper or ($CD4^{+}$) T-cells, that facilitate the activity of different types of immune responses by releasing different cytokines, 2) cytotoxic or ($CD8^{+}$) T-cells, in which they directly destroy virally or bacterially infected cells and cancer cells, 3) regulatory or (suppressor) T-cells, that are in charge for maintaining self-antigen tolerance and protecting against aberrant immune responses and autoimmune diseases (11,27,28).

Upon stimulation of TCR by the MHC molecule on the antigen-presenting cells, LCK, which is a member of SRC family kinases (SFK), phosphorylates the tyrosine residues of CD3 immunoreceptor tyrosine based activation motif (ITAM), followed by recruitment and binding of zeta-chain associated protein kinase of 70 KDa (ZAP-70) to phosphorylated ITAM. The activated ZAP-70, in turn, induces downstream signaling by phosphorylating linker for activation of T-cells (LAT), which promotes the aggregation of signaling components involving IL2- inducible T-cell kinase (ITK), phosphatidylinositol-3 kinase (PI3K), SH2 domain containing leukocyte protein of 76 kDa (SLP-76) and phospholipase Cγ1 (PLCγ1), followed by activation of several downstream signaling cascades leading to cell proliferation, activation and cytokine production (29-33).

1.3 B-LYMPHOCYTES

B-cells are one of the major sub-types of lymphocytes that originate from hematopoietic stem cells in bone marrow. They are an essential component of immune system and one of the key effectors of adaptive immunity (10). They are considered as positive regulators of immune responses, specialized in production of antibodies providing defense against different pathogens. Furthermore, B-cells are involved in other immunological functions including antigen presentation and cytokine secretion (34-36).

In humans, B-cells undergo several developmental stages. The first stage of the development that occurs in bone marrow is an antigen independent stage. This stage includes the differentiation of progenitor B-cell (pro-B-cells) to precursor B-cells (pre-B-cells), which further developed into immature B-cells. When the cells leave bone marrow and enter into the periphery, they become mature B-cells that co-express IgD and IgM molecules, and migrate to the lymphoid organs in which the second stage, antigen dependent stage, will start (37). This stage involves activation and further differentiation of mature B-cells into memory B-cells or antibody secreting plasma cells. B-cells that fail antigen recognition undergo programmed cell death, apoptosis (38).

B-cell receptor (BCR) signaling is crucial for B-lymphocytes proliferation, differentiation and survival. Functional BCR consists of two identical transmembrane immunoglobulin heavy chains (IgH) and two identical covalently associated light chains (IgL) paired with heterodimer co-receptor Igα (CD79α) and Igβ (CD79β) (39,40). The cytoplasmic tails of CD79 contains ITAM that is required for signal transduction (39,41).

BCR-antigen binding induces the SFKs to phosphorylate the tyrosine residues within ITAMs, which followed by formation of signaling complex of multiple protein tyrosine kinases and adaptor proteins, involving PI3K, B-cell linker protein (BLNK), spleen tyrosine kinase (SYK), BTK and PLCγ2, followed by PKC activation and calcium

mobilization, leading to activation of different transcription factors that induce B-cell proliferation, differentiation and antibody production (42-47).

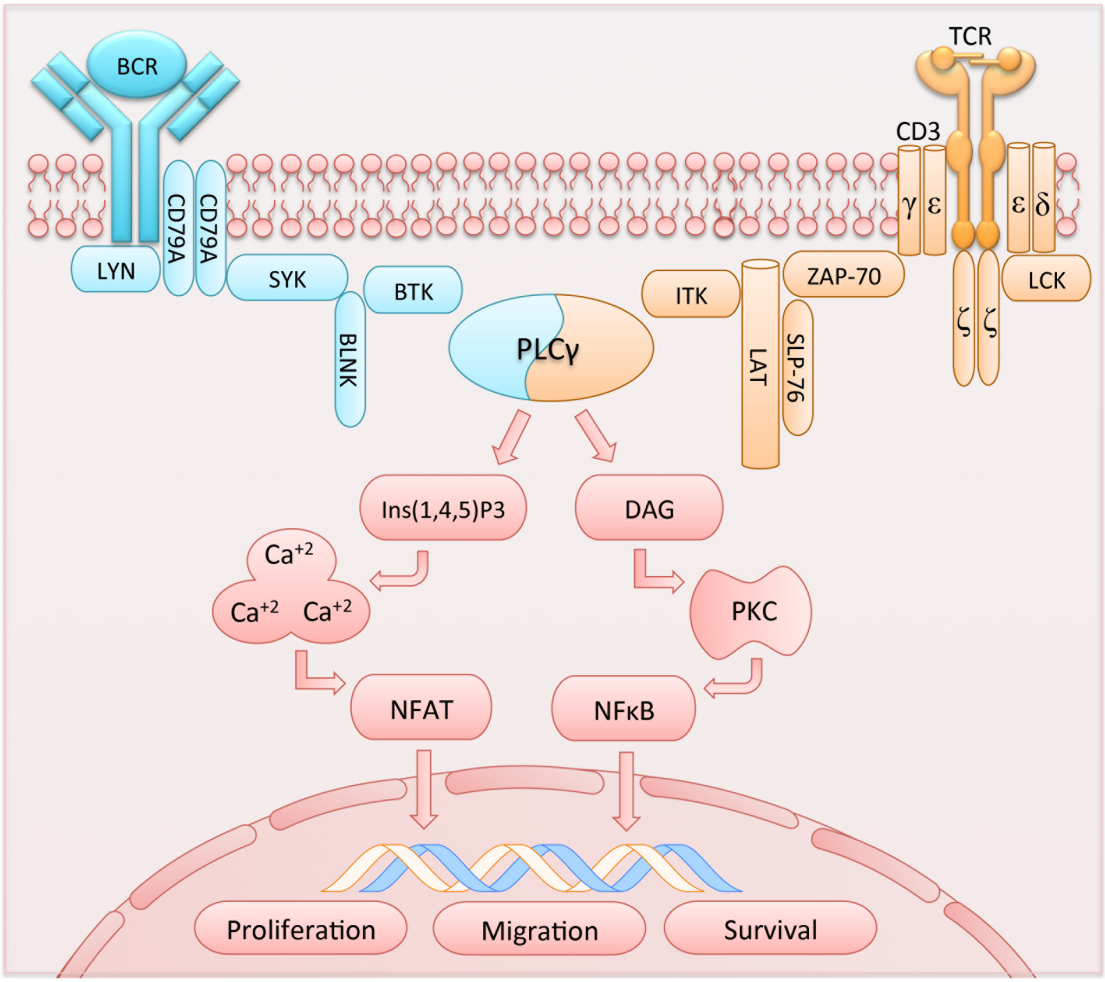

Figure 1. A simplified model of BCR/TCR signaling. BCR components are depicted in blue and TCR components are depicted in orange.

1.4 PROTEIN TYROSINE KINASES

1.4.1 Bruton's Tyrosine Kinase (BTK)

BTK is a cytoplasmic non-receptor tyrosine kinase, where the first clinical correlation has been identified in 1993, belonging to TEC family kinases (TFK) (48). BTK is constitutively expressed in most hematopoietic cells including both lymphoid and myeloid lineages (except plasma cells and T-cells) (49-51). Expression of BTK starts from very early stages of B-cell maturation, and is detected in all stages prior to terminally differentiated plasma cell (51-53).

The *BTK* gene is located on the q arm of the X chromosome in the 21.3-22 region (54-56). The protein is composed of 659 amino acids with structure similar to other members of TEC family kinases, consisting of five structural domains, which are essential for the regulation of various function of the protein. Starting with Pleckstrin homology (PH) and Tec homology (TH) domains at the N-terminus, that mediate plasma membrane binding, followed by Src homology 3 (SH3), for association with other proteins, Src homology 2 (SH2), for interaction with phosphorylated tyrosine residues, and kinase (SH1) domain in the C-terminus (Figure 2) (57-59). The protein has molecular weight of 77 KDa.

BTK plays a pivotal role in the BCR signaling and it is critical for B-cell maturation and survival (60,61). Loss-of-functional mutations in *BTK* gene cause B-cell developmental defects, giving rise to a severe B-cell deficiency with lack of antibody production, leading to an immunodeficiency syndrome called X-linked agammaglobulinemia (XLA) (62). The disease was first described in 1952 by Ogden C. Bruton. Most of the affected patients are diagnosed in early childhood, and characterized by recurrent, severe bacterial infections, mostly respiratory tract and gastrointestinal infections, due to absence of serum immunoglobulins of all classes (63). A milder phenotype, X-linked immunodeficiency (Xid), was observed in mice, and caused by substitution of arginine 28 with cysteine in the PH domain of BTK (64,65).

Two signaling steps are required for complete BTK activation, PH domain mediated membrane localization, and SFK mediated trans-phosphorylation (66,67). Following BCR stimulation, the tyrosine residues of the CD79 ITAM are phosphorylated by the activated SFKs, LYN and FYN (42,68,69). These subsequently serve as docking sites for the SYK kinase. Concomitantly, PI3K also becomes activated and generates the cell membrane-bound phosphatidylinositol 3,4,5- trisphosphate (PIP3), leading to PH domain mediated membrane translocation of BTK (70,71). The membrane-localized BTK is trans-phosphorylated by SFKs at tyrosine 551 in the catalytic region, which induces the auto-phosphorylation of tyrosine 223 residue in the SH3 domain. Activated BTK phosphorylates its direct substrate, PLCγ2 at Y753 and Y759, which hydrolyzes PIP2 into IP3 and DAG (44,67). This will lead to Ca^{+2} mobilization and PKC activation, followed by induction of several transcription factors necessary for proliferation, maturation and survival of B-cell (72,73).

BCR signaling is likely the key driver that promotes progression of B-cell leukemias and lymphomas (74,75). Although, constitutively active mutants of BTK have not been reported in patients with B-cell malignancies, studies have shown the involvement of BTK dependent pathways in the pathogenesis of various B-cell malignancies (76,77).

Moreover, BTK also has a functional role in signaling of chemokine receptor pathways that involve in tumor cell migration and homing, such as CXCR4, CXCR5 (78-80). This explains the redistribution of malignant cells from the lymphoid tissue into the peripheral blood following treatment of patients with BTK inhibitor. In addition, BTK is involved in chemokines CCL3, CCL4 secretions by malignant cells, and regulates integrin-mediated interaction of tumor cells with the microenvironment (81,82).

The involvement and central role of BTK in pro-survival pathways and microenvironment survival signals of tumor cells provide a general rationale for developing small molecule inhibitors targeting BTK (83).

1.4.1.1 FIRST GENERATION BTK INHIBITOR

1.4.1.1.1 Ibrutinib

In 2007, Pan and co-workers at Pharmacyclics synthesized a series of small molecule inhibitors of BTK. Because of the high efficacy and specificity for inhibiting BTK in preclinical models, the compound known as PCI-32765 or ibrutinib, was selected among the different compounds for further clinical trials (84). This compound showed encouraging clinical activities in various B-cell lymphomas and soon became the first US Food and Drug Administration (FDA) approved BTK inhibitor for the treatment of several lymphoproliferative malignancies (85-87).

Ibrutinib (imbruvica) is a first-in-class, orally administered, potent, selective, irreversible inhibitor of BTK with half maximal inhibitory concentration (IC_{50}) of about 0.5 nM/L (84,88). The drug abrogates the enzymatic activity of BTK by forming an irreversible covalent bond with cysteine-481 in the ATP binding site, thereby blocking BCR signal transduction (89-91). Ibrutinib has demonstrated impressive clinical efficacy in a variety of B-cell malignancies, and is now approved in the United States and in Europe for the treatment of CLL, MCL and WM (92-95). Moreover, the drug also shows promising and encouraging preclinical results against inflammatory and autoimmune diseases (85,96).

Ibrutinib is rapidly absorbed and eliminated after oral administration, reaching maximum serum concentration in 1-2 hours (92). The drug has short half-life (2-3 h), but because of its covalent binding to BTK, it can be given once daily, with BTK active-site occupancy at the dose of 420 mg, which remains covalently bound to BTK for 24 h (92,97).

Ibrutinib is generally well tolerated, and usually associated with grade 1-2 side effects, such as diarrhea, nausea, dyspnea, bleeding, fatigue and infections, that are self-limited and not required therapeutic intervention (98,99). Grade 3-4 toxic effects also reported in small population of patients, including, anemia, neutropenia and thrombocytopenia (99). These side effects could result from off-target effects of ibrutinib, since the drug

could also bind and inhibit other tyrosine kinases that have similar cysteine residue in the ATP binding site, including TEC, ITK, TXK, BMX, HER2, HER4, EGFR, BLK and JAK3 (91,100,101).

Additionally, the drug also affects other proteins that do not contain the cysteine residue, such as HCK, LCK, BRK, CSK and FRG, which might also contribute to the efficacy or toxicity of ibrutinib (85,101).

1.4.1.1.2 RESISTANCE MECHANISMS FOR IBRUTINIB

The ability of the malignant cells to adapt and develop resistance against targeted therapies is the main problem that limits the effectiveness of the treatment. Despite the clinical effectiveness of ibrutinib in multiple B-cell malignancies, not all the patients are responding to the drug, and small subset of patients treated with the drug have developed resistance (Figure 3) (102).

Mutation is the most potential mechanism of primary or acquired resistance to ibrutinib. These mutations mostly affect BTK or its direct downstream substrate PLCγ2 (103). The replacement of cysteine 481 with serine residue in BTK is the most common mutation that has been identified in the resistant patients with CLL or MCL (21,104,105). This will disrupt the covalent binding of ibrutinib, converting the irreversible inhibition of BTK to reversible inhibition, and decrease ibrutinib's affinity for BTK (106).

In addition to the BTK C481S mutation, a variety of acquired activating mutations in PLCγ2 have also been detected in mutation-prone patients with CLL. These gain-of-function mutations of PLCγ2 will bypass BTK entirely and induce activation of BCR downstream signaling that is not inhibited by ibrutinib (107,108).

Moreover, other BTK mutations like (C481F, C481R, C481Y, T474S, T474I and L528W) that are associated with BTK C481S or PLCγ2 variations have also been implicated in rare cases (107,109).

Recently, a study has reported the identification of a novel mutation (T316A) in the SH2 domain of BTK in ibrutinib resistant CLL patient, which is the first reported BTK mutation outside the kinase domain. However, how this mutation alters the drug binding is not well understood (110).

Interestingly, ibrutinib-resistant patients with DLBCL were associated with mutations that activated MYD88 or CARD11, whereas CXCR4 mutations confer resistance to ibrutinib in WM (111-113).

1.4.1.2 SECOND GENERATION BTK INHIBITORS

1.4.1.2.1 Acalabrutinib

Due to the off-target unwanted effects as well as development of resistance to ibrutinib, second-generation BTK inhibitors with enhanced selectivity and retained efficacy against mutated BTK have been developed (114,115).

Acalabrutinib (ACP-196) is a new selective, highly potent, more specific, and irreversible second-generation BTK inhibitor, which also binds covalently to cysteine-481 in the kinase region (115,116). The drug was designed to have improved target specificity and efficacy compared to ibrutinib (117). Only one case of acalabrutinib resistance has been observed so far, the patient had BTK C481S and PLCγ2 L845F resistant mutants (115). Also other BTK inhibitors such as CC-292, ONO/GS-4059 and BGB-3111 are being evaluated clinically for the treatment of hematologic malignancies (48,118).

1.4.2 IL-2-inducible T-cell kinase (ITK)

ITK, also known as EMT (expressed in mast cells and T lymphocytes), is a member of TFKs that expressed predominantly in T-lymphocytes along with Natural Killer cells and Mast cells (119,120). The kinase plays a major role in T-cell receptor signaling and it is crucial for the T-lymphocyte development, activation and survival (121-123). Loss-

of-function mutations of ITK cause severe, frequently fatal Epstein–Barr virus (EBV) infections (124).

Similar to other TFKs, ITK structure contains a C-terminal kinase domain, preceded by SH2, SH3 and TH domains, in addition to the N-terminal PH domain (Figure 2) (58,125). Upon T-cell receptor stimulation, ITK is translocate to the cell membrane via its PH domain, and subsequently trans-phosphorylated on its kinase domain tyrosine 511 residue by SFK, LCK. ITK then induces tyrosine phosphorylation of its downstream substrate, PLCγ1. Activated PLCγ1 hydrolyzes PIP2 into the second messengers IP3 and DAG, that induce Ca^{+2} mobilization thereby activation of nuclear factor of activated T-cells (NFAT) and nuclear factor kappa B (NFκB) transcription factors (29,126,127).

Many inflammatory and autoimmune diseases are mediated by aberrant activation of T-cells, and as a key molecule in TCR signaling, ITK became an attractive therapeutic target in these diseases. Preclinical *in vivo* studies have been started using ITK inhibitors for the treatment of allergic asthma and other inflammatory diseases (128-130).

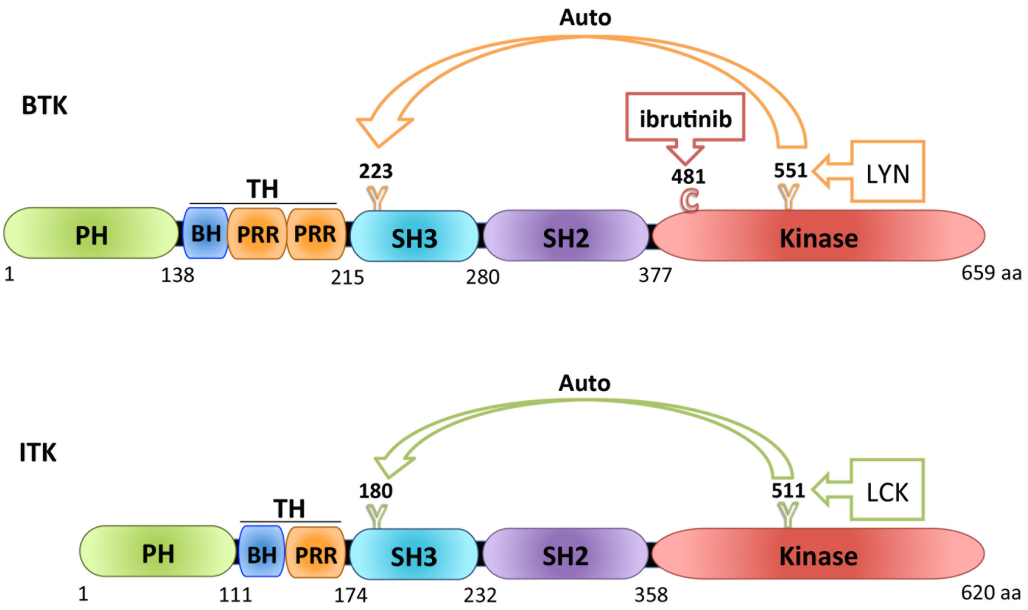

Figure 2. Schematic representation of ITK and BTK domain structures, showing the regulatory phosphorylation sites.

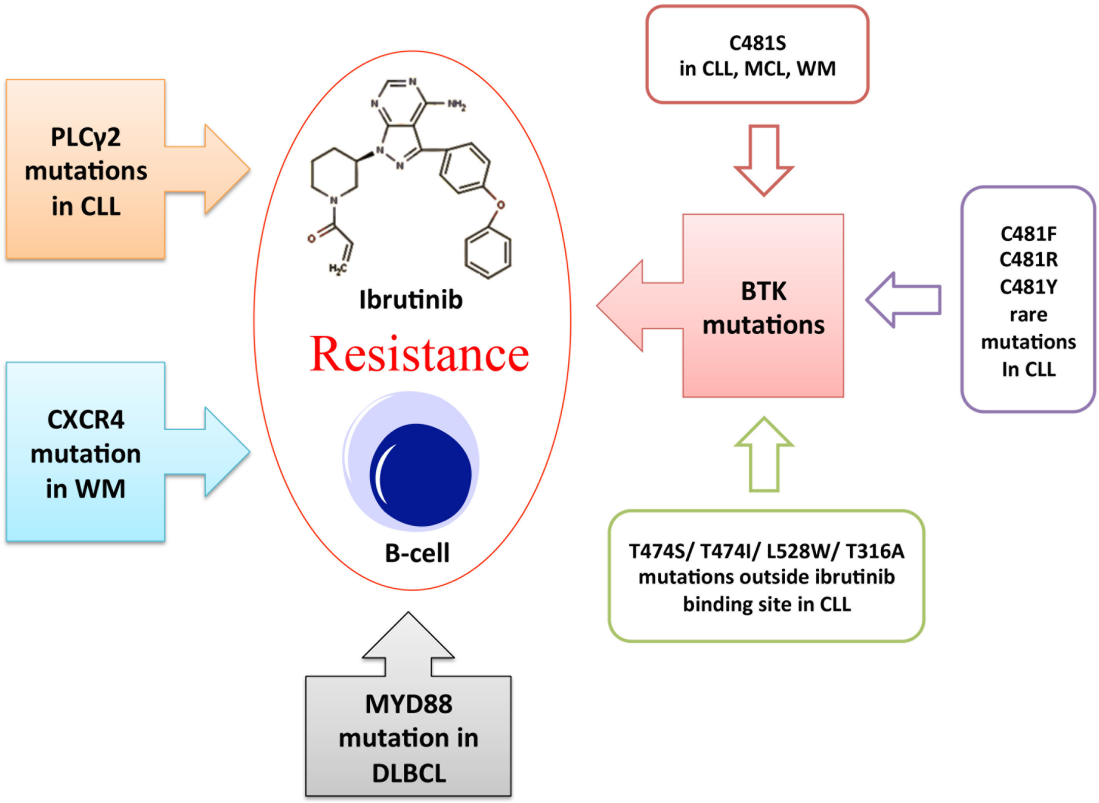

Figure 3. A model illustrating different ibrutinib resistance mechanisms in B-cell malignancies.

1.4.3 Spleen tyrosine kinase (SYK)

The non-receptor tyrosine kinase SYK together with its homologous, ZAP-70 are the only two members of the SYK/ZAP-70 family kinase (131). SYK showed a wide distribution pattern, the kinase is highly expressed by lymphocytes and myeloid cells. It is also expressed by non-hematopoietic cells, such as hepatocytes, neuronal cells, osteoclasts and epithelial cells (131-133). SYK deficient mice die prenatally due to an extensive hemorrhage caused by a defect in vascular tissue (134).

SYK possesses two N-terminal SH2 domains for association with other proteins, that are connected by inter domain A, and they separated from C-terminal catalytic region by inter domain B (Figure 4) (135,136).

SYK is crucial for signal transduction in different cell types and has diverse roles in immune and non-immune biological events. The kinase is essential for signaling via classical immunoreceptors (BCR, TCR and Fc receptor (FcR)), and induces further downstream signaling events leading to cytokine production, antibody production, cell proliferation, differentiation and survival (137,138). Moreover, it has been implicated in antifungal immunity through CLEC-7A-SYK-CARD9 pathway activation and IL-1β production in response to fungal infection (139,140).

Furthermore, SYK has been found to be a key molecule in several non-immune responses; including bone metabolism, which is required for osteoclast development and function. SYK-dependent signaling pathways are also important for platelet function as well as vascular development and integrity (141).

In resting cells, SYK retains a closed, auto-inhibitory conformation (142). Upon immunoreceptor engagement, SFK phosphorylate ITAM tyrosine residues; this follows by translocation of SYK to plasma membrane through binding of its N-terminal SH2 domains to the phosphorylated ITAM (143). Binding to ITAM abolishes the auto-inhibitory state and induces SYK phosphorylation at key tyrosines in the linker-region (Y352) and activation loop (Y525/526), which are crucial for kinase activation (144,145). SYK mediated signal transduction cascades recruit several kinases and adaptor proteins that initiate downstream responses, such as VAV, BLNK, PI3K and PLCγ2 (146,147). SYK activity is tightly controlled, and several regulatory molecules are involved in the negative regulation of SYK, such as protein tyrosine phosphatases SHP1 and PTPROt, and the E3 ubiquitin ligase CBL, which regulates SYK expression (148-150).

An alternatively spliced shorter variant of SYK (SYK-B) has been detected in mouse, rat and human cells. The two isoforms share the major structural domains, but SYK-B lacks 23 amino acid insert in the inter domain B, including nuclear localization signals (151). Therefore, they show differential subcellular localization. Unlike full length SYK that localizes both in the nucleus and cytoplasm, the shorter isoform is predominantly cytoplasmic (152,153).

SYK may function as an oncogene and contributes in growth and survival of various hematological malignancies, since over expression or deregulated SYK activity has been observed in different B-cell and T-cell neoplasms, such as CLL, MCL, DLBCL, follicular lymphoma (FL) and PTCL (154-157). Moreover, involvement of SYK in two different chromosomal translocation events, leads to expression of enzymatically active fusion kinases, ITK-SYK, in PTCL and TEL-SYK, in myelodysplastic syndrome, further supporting the possible oncogenic role of SYK in hematological malignancies (18,158).

Notably, SYK has been reported as tumor suppressor in multiple non-hematopoietic cancer types. Several clinical studies have shown that the down-regulation of SYK in breast cancer, gastric cancer and melanoma correlates with poor prognosis of the disease (159). However, this relationship remains elusive.

Given the involvement of SYK in various signaling pathways in multiple immune and non-immune biological responses, and its role in the pathogenesis of malignant and inflammatory disorders, the kinase became a promising therapeutic target in those diseases (160). Fostamatinib, the orally administered SYK inhibitor that targets the conserved ATP binding site has shown potential therapeutic efficacy in the treatment of autoimmune diseases, and CLL (161,162).

1.5 SYK FUSION KINASES

1.5.1 ITK-SYK

PTCL are heterogeneous, highly aggressive non-Hodgkin's lymphomas with a high mortality rate arising from mature T-cells and infiltrating different organs such as lymph nodes, spleen, liver and skin. Among PTCLs, unspecified peripheral T-cell lymphoma (PTCLU) represents the most common subtype (163). Recurrent

chromosomal translocation and generation of active fusion proteins were found to be important driver of various malignancies. Novel t(5;9)(q33;q22) translocation was first described in 2006 as a recurrent genomic alteration in 17% of patients with PTCLU (18). This gene rearrangement involves *ITK* and *SYK* genes on the chromosomes 5 and 9 respectively. Consequently, the first 165 amino acids from the N-terminal PH and TH regions of ITK coupled with the 330 amino acids from C-terminal inter domain B and kinase domain of SYK, yielding a chimeric oncogene ITK-SYK (495 amino acids) (Figure 4), which is believed to be the cause of the disease (164,165).

Biochemical and functional studies have shown that ITK-SYK is a constitutively active tyrosine kinase, which phosphorylates various downstream signaling proteins like SLP-76, PLCγ1, AKT and ERK, and induces CD69 up-regulation and IL-2 production in T-cells (166-168). *In vivo* expression of the fusion kinase induces T-cell transformation and yielding aggressive T-cell lymphoma with phenotype similar to human disease (169). The transforming capacity of ITK-SYK is kinase dependent.

In mice, T-cell specific expression of ITK-SYK causes T-cell lymphoproliferation with systemic inflammation (169). The onset of the disease is dependent on the expression level of the oncogene. The low expression of ITK-SYK leads to the development of aggressive disease within few weeks, while high expression of the fusion kinase will cause delayed disease development (20-27 weeks), since the high expression of the fusion kinase activates Blimp-1 dependent terminal differentiation of the T-cells (170). Surprisingly, B-cell specific expression of ITK-SYK did not induce B-cell malignancy, but led to delayed T-cell lymphoma.

Moreover, *in vivo* studies have shown that SYK inhibitor R406 could potently inhibit ITK-SYK-induced T-cell lymphoproliferative disease in mice, which suggests that ITK-SYK could be a suitable therapeutic target for the treatment of the patients carrying the fusion protein (169).

ITK-SYK localizes into different compartments of the cell. Some studies demonstrated that ITK-SYK localizes in the plasma membrane, and the membrane localization of the kinase is crucial for its transforming potential (167). Interestingly, cytoplasmic localized ITK-SYK R29C variant that compromises membrane recruitment, induces more aggressive T-cell lymphoma in the transplanted mice (171).

SYK contains a number of regulatory tyrosines in the inter domains A, B and kinase domain. ITK-SYK retains most of these tyrosine sites, and it is constitutively phosphorylated on both activation loop and linker region tyrosine sites that are required for SYK catalytic activity (172). However, there are conflicting results about the role of these tyrosine sites in the activity and regulation of ITK-SYK, since it has been shown that ITK-SYK is lacking the auto-inhibitory conformation and the open conformation structure of the fusion kinase is responsible for the constitutive kinase activity (172).

1.5.2 TEL-SYK

ETV6, also known as TEL (Translocation-ETS-Leukemia), is a transcriptional repressor belonging to the erythroblast transformation specific (ETS) family, and expresses in a wide variety of cells (173). Chromosomal translocations involving TEL have been identified in different hematological malignancies, leading to fusion of TEL with different protein kinases, such as TEL-JAK2, TEL-TRKC, TEL-ARG, TEL-ABL TEL-PDGFβR and TEL-SYK (173-177).

The t(9;12)(q22;p12) translocation yielding TEL-SYK fusion kinase was reported in a patient with myelodysplastic syndrome that progressed later to AML (158). The TEL part of TEL-SYK fusion contains the N-terminal pointed (PNT) domain linked to C-terminal linker region and complete kinase domain of SYK (Figure 4) (178). TEL-SYK acquires constitutive activation through PNT domain-mediated oligomerization and subsequent auto-phosphorylation (179). TEL-SYK induces IL-3 independent growth and proliferation of BAF3 cells, causing activation of PI3K, MAPK and constitutive tyrosine phosphorylation of STAT5 (179). *In vivo*, overexpression of the fusion kinase in murine pre-B-cells leads to lymphoid leukemia in mice (178,180).

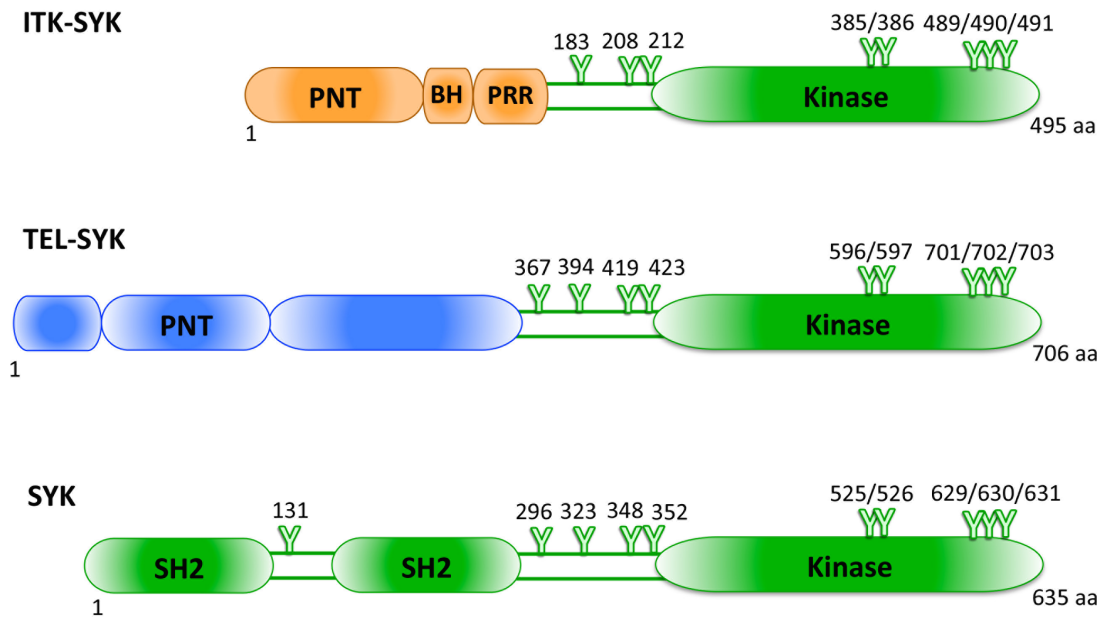

Figure 4. Schematic representation of SYK, ITK-SYK and TEL-SYK domain structure, showing the corresponding tyrosine sites.

1.6 PRIMARY IMMUNODEFICIENCIES (PIDs)

PIDs comprise a heterogeneous group of genetically inherited disorder, which are characterized by defective immune responses. These diseases can be inherited in different patterns, most commonly by autosomal recessive or X-linked pattern of inheritance (181). The defective genes encode destabilized or activity-reduced important proteins affecting the function of immune system (182,183).

Generally, the clinical symptoms of these diseases include severe recurrent infections, auto-inflammatory disorders and allergy (184). Furthermore, hematologic and non-hematologic malignancies were often reported with increased frequency as a complication of many PIDs (185).

There are over 200 different disorders and more than 200 genetic mutations that have

been identified in patients with PIDs (183,186). The severe diseases have an overall prevalence of approximately 1:10000 individuals, and they are categorized by IUIS into 9 general categories depending on the nature of immune defect, including defects in innate immunity, predominantly antibody deficiencies, diseases of immune dysregulation, auto-inflammatory disorders, combined immunodeficiencies, combined immunodeficiencies with associated or syndromic features, congenital defects of phagocyte number, function, or both, complement deficiencies and phenocopies of PID (187,188).

1.7 Phosphoglucomutase-3 (PGM3)

Glycosylation is a posttranslational modification in which the proteins are modified by attachment of sugar moieties to specific amino acid residues. The process is critical for the proper folding of the protein, which affects its function and stability. The main two types of the glycosylation are N-glycosylation, by which the sugar molecule is attached to the amino group of the asparagine side chain, and O-glycosylation, by which the carbohydrate is attached to the hydroxyl group of serine or threonine (182,189,190).

Glycosylation has a key role in various biological processes, including cell adhesion, migration and immune responses. Defect in glycosylation will lead to an immune disease, which associate with skeletal and neurological abnormalities called congenital disorder of glycosylation (CDG). Moreover, glycosylation defects have also been described to cause PIDs (191,192).

PGM3 or N-acetyl glucosamine-phosphate mutase (AGM1) is an enzyme belonging to the hexose-phosphate mutase family that catalyzes the conversion of N-acetyl-glucosamine-6-phosphate to N-acetyl-glucosamine-1-phosphate, an important step in the hexosamine biosynthesis pathway for the generation of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc). The latter is an essential component for protein glycosylation (193,194).

PGM3 is widely expressed in cells of different organs such as bone marrow, liver, pancreas and testis. Autosomal recessive mutations affecting *PGM3* gene have been reported in 23 immunodeficient patients leading to impairment of the protein expression and enzymatic function, resulting in the disruption of the glycosylation (Figure 5)(195-198).

Patients with hypomorphic PGM3 mutations display similar clinical features, including recurrent bacterial, viral and fungal infections associated with allergy and atopic disease, in addition to connective tissue and neurologic abnormalities and marked elevation of serum IgE level in the majority of the patients (199,200). Moreover, it has been reported that complete loss of PGM3 in mice is embryonically lethal (193).

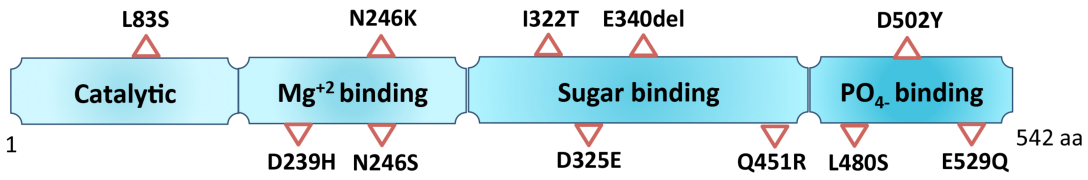

Figure 5. Domain structure of PGM3 showing the detected mutation sites.

2 AIMS

The objectives of this study are:

- To characterize the abnormalities in TEC family kinases.
- To characterize various mutations of BTK at C481 that can cause ibrutinib resistance.
- To characterize the ITK-SYK fusion protein using biochemical and molecular cell biology methods.
- To compare ITK-SYK activation to that of the TEL-SYK fusion protein.
- To study the mutations in the PGM3 gene which cause primary immunodeficiency.

3 MATERIALS AND METHODS

3.1 CELL CULTURING

3.1.1 Cell lines

Jurkat (human T-cell leukemia cell line) BAF3 (Bone marrow-derived IL-3-dependent pro-B), DT40 (chicken lymphoma cells), NIH3T3 (mouse embryonic fibroblast), SYF (mouse cells deficient in Src, Yes and Fyn), HEK-293T (human embryonic kidney cells) and COS-7 (African green monkey fibroblast-like kidney) cell lines were purchased from the American Type Culture Collection (ATCC). The B7.10 cell line was donated by Dr. T. Kurosaki's laboratory, Japan.

3.1.2 Primary cells

Peripheral blood from the patient and healthy controls were collected in EDTA tubes. The B-cells from collected samples were subsequently transformed with EBV, then they expanded and cultured in RPMI 1640 containing 50 mg/ml Gentamicin with 20% bovine serum.

3.2 TRANSFECTION AND ELECTROPORATION

In this work, two different protocols have been used for transient transfection of the plasmids. Polyethylenimine (PEI) has been used for the transfection of the adherent cells. All other hematopoietic cells were transfected by using the Neon transfection system, using different setting for each cell line according to the manufacture's protocol.

3.3 PROTEIN ANALYSIS

3.3.1 Western blot

Western blot or immunoblot is an important and widely used technique that allows the separation and detection of proteins in naïve or phosphorylated states. The fresh made

RIPA buffer containing protease and phosphatase inhibitors have been used to facilitate cell lysis. Then proteins in the lysate were separated depending on their size by gel electrophoresis and subsequently transferred from the gel onto nitrocellulose membranes, followed by incubation with proper primary and secondary antibodies. The membranes were scanned using Odyssey Imager from LI-COR Biosciences GmbH.

3.3.2 Immunoprecipitation

Immunoprecipitation is one of the most frequently used methods to isolate and purify a particular protein out of the heterogeneous mixture of different proteins, by incubating the specific antibody that binds to the targeted protein within the protein mixture. Then the antibody-protein complex is pulled down by using protein A or G beads, which enable the isolation of desired protein from the rest of the mixture. Western blot or other assay techniques can be used for the detection of the precipitated protein. The technique is also used to detect protein-protein interactions.

3.3.3 Immunofluorescence microscopy

Immunofluorescence is a common laboratory technique used to analyze the expression and distribution of the interested protein or other molecules within the cell or tissue section by using a specific fluorescently labeled antibodies.

The transfected cells were fixed, permeabilized and stained using two antibodies, the primary unlabeled antibody that form an immune complex with the targeted protein, and fluorophore-conjugated secondary antibody that directed against the primary antibody. Images were captured using Olympus microscope (Olympus-IX81), and the images were processed using Cell Sense Dimension software (Olympus, Tokyo, Japan).

3.4 *IN VITRO* KINASE ASSAY

In vitro kinase assay is a specific technique in which the enzymatic activity of a kinase is measured in purified form without cell lysates. In this method the kinase is incubated

with the targeted substrate in the presence of ATP in the kinase reaction buffer, in which the kinase will transfer the phosphate group from ATP onto the substrate. In our study, the immunoprecipitated BTK, or the BTK mutants, were mixed with immunoprecipitated PLCγ2, and the mixture is incubated in the kinase buffer in the presence of ATP for 30 minutes at 30 °C.

3.5 HEALING ASSAY

The wound-healing assay has been used to study the effect of expression of genes or the addition of chemical compounds on the proliferation and migration rates of cells in vitro. We used μ-Dish 35 mm, high, with culture insert from ibidi for wound healing assay. Cells were transfected with the plasmids, then after 24 hours a suspension of the transfected cells were placed in both wells of the silicon-insert, and incubated for additional 24 hours at 37 °C and 5% CO_2. Then the silicon-insert was removed by using sterile tweezers, and a cell free gap of 500 μm was created, and cell images were captured at regular intervals using 4X dry objective under fluorescence microscope.

3.6 NUCLEAR AND CYTOPLASMIC FRACTIONATION

Nuclear and cytoplasmic fractionation is a commonly used method for the isolation of cytoplasmic and nuclear fractions in order to determine the localization of the proteins within the cell. We have studied the subcellular localization of SYK fusion kinases in the Jurkat cells by using NE-PER nuclear and cytoplasmic extraction reagent kit (Thermo Scientific).

4 RESULTS AND DISCUSSIONS

4.1 PAPER I

Substitution scanning identifies a novel, catalytically active ibrutinib-resistant BTK cysteine 481 to threonine (C481T) variant

Minorities of patients treated with ibrutinib have developed resistance to the drug. Resistance to ibrutinib often involves mutation of the cysteine 481 residue in the BTK catalytic domain, where ibrutinib binding occurs.

In this study, we generated various variants of BTK mutated at cysteine 481 site and we have studied the activity and ibrutinib sensitivity of the generated mutants. Six different BTK mutants were generated by replacing cysteine 481 with (phenylalanine, tryptophan, arginine, glycine, tyrosine, serine), following single nucleotide substitutions of the codon. We have also generated cysteine 481 to threonine replacement, which required exchange of two nucleotides.

According to the functional assays, the kinase activity of BTK is completely lost when C481 is substituted by phenylalanine, tyrosine, tryptophan or arginine due to the big and bulky structures of these amino acids, which cause structural clashes within the kinase domain. Substitution with glycine that lacks the polar side chain, which is important for functional interaction within kinase domain, severely impaired the kinase activity.

Substitutions of cysteine 481 by serine or threonine were the only replacements with retained kinase activity. Furthermore, since these variants cannot covalently bind to ibrutinib, both would likely be of clinical importance in the development of drug resistance. We therefore predict that not only serine, but also threonine escape mutants,

not reported to date, could occur in patients treated with BTK inhibitors binding covalently to cysteine 481. However, resistance due to threonine substitution is expected less frequently as compared to serine, since replacement with threonine would require exchange of two nucleotides.

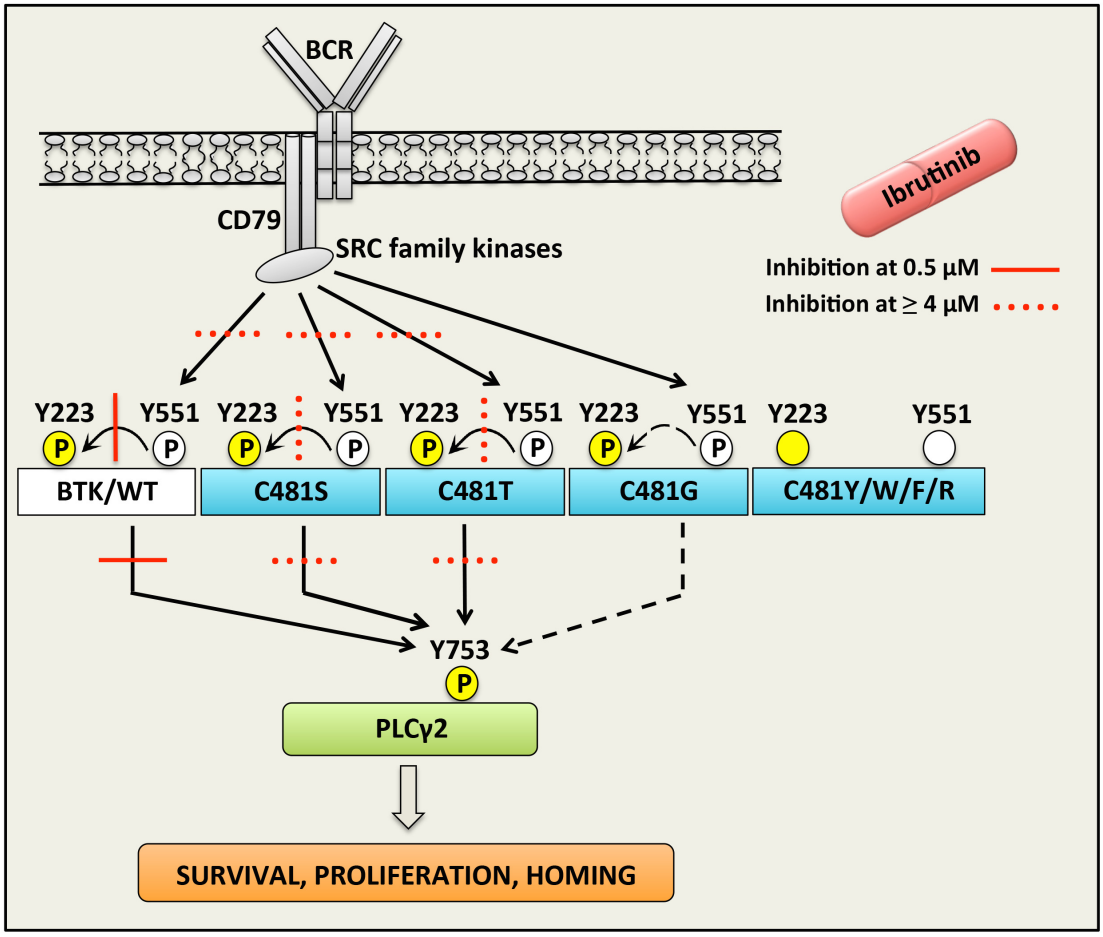

Figure 6. Schematic view of BCR signaling pathway illustrating the ibrutinib sensitivity of BTK C481 mutants and their effect on downstream signaling (201).

4.2 PAPER II

Differential regulatory effects of N-terminal region in SYK-fusion kinases reveal unique activation-inducible nuclear translocation of ITK-SYK

In this study, we have compared the role of N-terminal region domains for the regulation of SYK fusion kinases in terms of phosphorylation, activation, stability and localization. We also investigated the effect of these rearrangements on the degree of modularity of the domain by studying the functional activity of PH-TH domain in ITK-SYK and BTK-SYK and PNT domain in TEL-SYK.

We have compared the proliferation and the migration rate, which reflect the oncogenic potential of these fusion kinases, by using a wound-healing assay. The healing process and the wound gap filling were significantly stronger upon TEL-SYK expression compared to ITK-SYK and BTK-SYK expression. We also compared the subcellular localization of the fusion kinases and found that BTK-SYK and TEL-SYK are localized predominantly in perinuclear region and cytoplasm respectively. Interestingly, ITK-SYK showed robust activation-mediated nuclear translocation in COS-7 and Jurkat cells, using two different methods, western blotting and fluorescence microscopy.

Subsequently, we investigated the activity of the loss of function PH-domain mutant ITK-SYK-R29C and BTK-SYK-R28C. Both mutants had retained kinase activity and they potently phosphorylated their substrate, SLP-76. In summary, we found that the SYK fusion kinases are constitutively active and behave differently from each other, although they shared the same kinase domain of SYK, seemingly reflecting the differences of the N- terminal region.

4.3 PAPER III

Susceptibility to infections, without concomitant hyper-IgE, reported in 1976, is caused by hypomorphic mutation in the phosphoglucomutase 3 (PGM3) gene

In this study, we have described a new homozygous mutation in the *PGM3* gene replacing isoleucine 322 with threonine, in a family with immunodeficient sibling. Patients with the identified PGM3 mutation displayed recurrent airway infections, varicella, pneumonia and allergy.

The laboratory analysis showed that the patient carrying PGM3 mutation had elevated level of eosinophil with reduced neutrophil and lymphocyte counts. The patient had increased serum IgA and normal IgE level.

We have also studied the stability of the mutated protein and we found that the EBV transformed patient B-cells grew much slower comparing to the control cells. Moreover, the immunoblotting result showed that the I322T mutated PGM3 is instable and the mutation induces protein degradation. Then we performed functional analysis for the protein, and we found that this substitution effectively reduced PGM3 enzymatic capacity.

5 CONCLUSIONS

These studies have shown that:

Paper I:

- BTK with threonine substitution at cysteine 481 maintained catalytic activity.
- Since the C481T variant cannot covalently bind to ibrutinib, it would likely be of clinical importance for the development of drug resistance.
- Resistance due to threonine substitution is expected less frequently as compared to serine, since replacement with threonine would require exchange of two nucleotides.
- C481Y, C481F, C481W and C481R variants of BTK are catalytically inactive and they predicted to be very scarce and they require compensatory mutations.

Paper II:

- SYK fusion kinases are constitutively active and behave differently from each other.
- Activation of SYK fusion kinases is not only dependent on the presence of intact PH-TH or PNT domains, but also on the absence of an auto inhibitory conformation.
- ITK-SYK robustly translocates to the nucleus upon activation.

Paper III:

- I322T substitution mutation in PGM3 is a cause of immunodeficiency disease.
- The mutated protein is severely destabilized and impaired the enzymatic activity.

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7 REFERENCES

1. Pearson, H. (2006) Genetics: what is a gene? *Nature* **441**, 398-401
2. Pennisi, E. (2007) Genomics. DNA study forces rethink of what it means to be a gene. *Science* **316**, 1556-1557
3. Crick, F. H., Barnett, L., Brenner, S., and Watts-Tobin, R. J. (1961) General nature of the genetic code for proteins. *Nature* **192**, 1227-1232
4. Jacob, F., and Monod, J. (1961) Genetic regulatory mechanisms in the synthesis of proteins. *J Mol Biol* **3**, 318-356
5. Nachman, M. W., and Crowell, S. L. (2000) Estimate of the mutation rate per nucleotide in humans. *Genetics* **156**, 297-304
6. Balmain, A. (2001) Cancer genetics: from Boveri and Mendel to microarrays. *Nat Rev Cancer* **1**, 77-82
7. Rowley, J. D. (2001) Chromosome translocations: dangerous liaisons revisited. *Nat Rev Cancer* **1**, 245-250
8. Ogawa, M. (1993) Differentiation and proliferation of hematopoietic stem cells. *Blood* **81**, 2844-2853
9. Saunders, A. M. (1980) White blood cells: what to do beyond measurement. *Blood Cells* **6**, 357-364
10. LeBien, T. W., and Tedder, T. F. (2008) B lymphocytes: how they develop and function. *Blood* **112**, 1570-1580
11. Appay, V., van Lier, R. A., Sallusto, F., and Roederer, M. (2008) Phenotype and function of human T lymphocyte subsets: consensus and issues. *Cytometry A* **73**, 975-983
12. Dalla-Favera, R. (2012) Lymphoid malignancies: many tumor types, many altered genes, many therapeutic challenges. *J Clin Invest* **122**, 3396-3397
13. Magic, Z., Novkovic, T., Cikota, B., Tasic-Radic, O., Tarabar, O., and Stamatovic, D. (2005) Genetic alterations in B-cell non-Hodgkin's lymphoma. *Vojnosanit Pregl* **62**, 87-96
14. Mullighan, C. G. (2013) Genome sequencing of lymphoid malignancies. *Blood* **122**, 3899-3907
15. Re, D., Zander, T., Diehl, V., and Wolf, J. (2002) Genetic instability in Hodgkin's lymphoma. *Ann Oncol* **13 Suppl 1**, 19-22
16. Van Vlierberghe, P., and Ferrando, A. (2012) The molecular basis of T cell acute lymphoblastic leukemia. *J Clin Invest* **122**, 3398-3406
17. Mullighan, C. G. (2012) Molecular genetics of B-precursor acute lymphoblastic leukemia. *J Clin Invest* **122**, 3407-3415

18. Streubel, B., Vinatzer, U., Willheim, M., Raderer, M., and Chott, A. (2006) Novel t(5;9)(q33;q22) fuses ITK to SYK in unspecified peripheral T-cell lymphoma. *Leukemia* **20**, 313-318

19. Pasqualucci, L. (2013) The genetic basis of diffuse large B-cell lymphoma. *Curr Opin Hematol* **20**, 336-344

20. Yang, G., Zhou, Y., Liu, X., Xu, L., Cao, Y., Manning, R. J., Patterson, C. J., Buhrlage, S. J., Gray, N., Tai, Y. T., Anderson, K. C., Hunter, Z. R., and Treon, S. P. (2013) A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in Waldenstrom macroglobulinemia. *Blood* **122**, 1222-1232

21. Woyach, J. A., Furman, R. R., Liu, T. M., Ozer, H. G., Zapatka, M., Ruppert, A. S., Xue, L., Li, D. H., Steggerda, S. M., Versele, M., Dave, S. S., Zhang, J., Yilmaz, A. S., Jaglowski, S. M., Blum, K. A., Lozanski, A., Lozanski, G., James, D. F., Barrientos, J. C., Lichter, P., Stilgenbauer, S., Buggy, J. J., Chang, B. Y., Johnson, A. J., and Byrd, J. C. (2014) Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N Engl J Med* **370**, 2286-2294

22. Anderson, G., Moore, N. C., Owen, J. J., and Jenkinson, E. J. (1996) Cellular interactions in thymocyte development. *Annu Rev Immunol* **14**, 73-99

23. Klein, L., Kyewski, B., Allen, P. M., and Hogquist, K. A. (2014) Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat Rev Immunol* **14**, 377-391

24. Stritesky, G. L., Jameson, S. C., and Hogquist, K. A. (2012) Selection of self-reactive T cells in the thymus. *Annu Rev Immunol* **30**, 95-114

25. Starr, T. K., Jameson, S. C., and Hogquist, K. A. (2003) Positive and negative selection of T cells. *Annu Rev Immunol* **21**, 139-176

26. Gascoigne, N. R., Rybakin, V., Acuto, O., and Brzostek, J. (2016) TCR Signal Strength and T Cell Development. *Annu Rev Cell Dev Biol* **32**, 327-348

27. Luckheeram, R. V., Zhou, R., Verma, A. D., and Xia, B. (2012) CD4(+)T cells: differentiation and functions. *Clin Dev Immunol* **2012**, 925135

28. Shevach, E. M. (2000) Regulatory T cells in autoimmmunity*. *Annu Rev Immunol* **18**, 423-449

29. van Leeuwen, J. E., and Samelson, L. E. (1999) T cell antigen-receptor signal transduction. *Curr Opin Immunol* **11**, 242-248

30. Palacios, E. H., and Weiss, A. (2004) Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. *Oncogene* **23**, 7990-8000

31. Salmond, R. J., Filby, A., Qureshi, I., Caserta, S., and Zamoyska, R. (2009) T-cell receptor proximal signaling via the Src-family kinases, Lck and Fyn, influences T-cell activation, differentiation, and tolerance. *Immunol Rev* **228**, 9-22

32. Leitenberg, D., Boutin, Y., Constant, S., and Bottomly, K. (1998) CD4 regulation of TCR signaling and T cell differentiation following stimulation with peptides of different affinities for the TCR. *J Immunol* **161**, 1194-1203

33. Bockenstedt, L. K., Goldsmith, M. A., Koretzky, G. A., and Weiss, A. (1987) The activation of T lymphocytes. *Rheum Dis Clin North Am* **13**, 411-430

34. Mauri, C., and Bosma, A. (2012) Immune regulatory function of B cells. *Annu Rev Immunol* **30**, 221-241

35. Lund, F. E. (2008) Cytokine-producing B lymphocytes-key regulators of immunity. *Curr Opin Immunol* **20**, 332-338

36. Janeway, C. A., Jr., Ron, J., and Katz, M. E. (1987) The B cell is the initiating antigen-presenting cell in peripheral lymph nodes. *J Immunol* **138**, 1051-1055

37. Li, Y. S., Hayakawa, K., and Hardy, R. R. (1993) The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver. *J Exp Med* **178**, 951-960

38. Tobon, G. J., Izquierdo, J. H., and Canas, C. A. (2013) B lymphocytes: development, tolerance, and their role in autoimmunity-focus on systemic lupus erythematosus. *Autoimmune Dis* **2013**, 827254

39. Tolar, P., Sohn, H. W., and Pierce, S. K. (2008) Viewing the antigen-induced initiation of B-cell activation in living cells. *Immunol Rev* **221**, 64-76

40. Hombach, J., Tsubata, T., Leclercq, L., Stappert, H., and Reth, M. (1990) Molecular components of the B-cell antigen receptor complex of the IgM class. *Nature* **343**, 760-762

41. Shinohara, H., and Kurosaki, T. (2006) Genetic analysis of B cell signaling. *Subcell Biochem* **40**, 145-187

42. Reth, M., and Wienands, J. (1997) Initiation and processing of signals from the B cell antigen receptor. *Annu Rev Immunol* **15**, 453-479

43. Monroe, J. G. (2006) ITAM-mediated tonic signalling through pre-BCR and BCR complexes. *Nat Rev Immunol* **6**, 283-294

44. Kurosaki, T. (1999) Genetic analysis of B cell antigen receptor signaling. *Annu Rev Immunol* **17**, 555-592

45. Kurosaki, T. (2002) Regulation of B-cell signal transduction by adaptor proteins. *Nat Rev Immunol* **2**, 354-363

46. Kurosaki, T., and Hikida, M. (2009) Tyrosine kinases and their substrates in B lymphocytes. *Immunol Rev* **228**, 132-148

47. DeFranco, A. L. (1997) The complexity of signaling pathways activated by the BCR. *Curr Opin Immunol* **9**, 296-308

48. Smith, C. I. (2016) From identification of the BTK kinase to effective management of leukemia. *Oncogene*

49. Vetrie, D., Vorechovsky, I., Sideras, P., Holland, J., Davies, A., Flinter, F., Hammarstrom, L., Kinnon, C., Levinsky, R., Bobrow, M., and et al. (1993) The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. *Nature* **361**, 226-233

50. Tsukada, S., Saffran, D. C., Rawlings, D. J., Parolini, O., Allen, R. C., Klisak, I., Sparkes, R. S., Kubagawa, H., Mohandas, T., Quan, S., and et al. (1993) Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* **72**, 279-290

51. Smith, C. I., Baskin, B., Humire-Greiff, P., Zhou, J. N., Olsson, P. G., Maniar, H. S., Kjellen, P., Lambris, J. D., Christensson, B., Hammarstrom, L., and et al. (1994) Expression of Bruton's agammaglobulinemia tyrosine kinase gene, BTK, is selectively down-regulated in T lymphocytes and plasma cells. *J Immunol* **152**, 557-565

52. Genevier, H. C., Hinshelwood, S., Gaspar, H. B., Rigley, K. P., Brown, D., Saeland, S., Rousset, F., Levinsky, R. J., Callard, R. E., Kinnon, C., and et al. (1994) Expression of Bruton's tyrosine kinase protein within the B cell lineage. *Eur J Immunol* **24**, 3100-3105

53. de Weers, M., Verschuren, M. C., Kraakman, M. E., Mensink, R. G., Schuurman, R. K., van Dongen, J. J., and Hendriks, R. W. (1993) The Bruton's tyrosine kinase gene is expressed throughout B cell differentiation, from early precursor B cell stages preceding immunoglobulin gene rearrangement up to mature B cell stages. *Eur J Immunol* **23**, 3109-3114

54. Kwan, S. P., Kunkel, L., Bruns, G., Wedgwood, R. J., Latt, S., and Rosen, F. S. (1986) Mapping of the X-linked agammaglobulinemia locus by use of restriction fragment-length polymorphism. *J Clin Invest* **77**, 649-652

55. Mensink, E. J., Thompson, A., Schot, J. D., van de Greef, W. M., Sandkuyl, L. A., and Schuurman, R. K. (1986) Mapping of a gene for X-linked agammaglobulinemia and evidence for genetic heterogeneity. *Hum Genet* **73**, 327-332

56. Kwan, S. P., Terwilliger, J., Parmley, R., Raghu, G., Sandkuyl, L. A., Ott, J., Ochs, H., Wedgwood, R., and Rosen, F. (1990) Identification of a closely linked DNA marker, DXS178, to further refine the X-linked agammaglobulinemia locus. *Genomics* **6**, 238-242

57. Smith, C. I., Islam, T. C., Mattsson, P. T., Mohamed, A. J., Nore, B. F., and Vihinen, M. (2001) The Tec family of cytoplasmic tyrosine kinases: mammalian Btk, Bmx, Itk, Tec, Txk and homologs in other species. *Bioessays* **23**, 436-446

58. Mano, H. (1999) Tec family of protein-tyrosine kinases: an overview of their structure and function. *Cytokine Growth Factor Rev* **10**, 267-280

59. Mohamed, A. J., Nore, B. F., Christensson, B., and Smith, C. I. (1999) Signalling of Bruton's tyrosine kinase, Btk. *Scand J Immunol* **49**, 113-118

60. Satterthwaite, A. B., Li, Z., and Witte, O. N. (1998) Btk function in B cell development and response. *Semin Immunol* **10**, 309-316

61. Khan, W. N. (2001) Regulation of B lymphocyte development and activation by Bruton's tyrosine kinase. *Immunol Res* **23**, 147-156

62. Smith, C. I. E., and Berglof, A. (1993) X-Linked Agammaglobulinemia. in *GeneReviews(R)* (Pagon, R. A., Adam, M. P., Ardinger, H. H., Wallace, S. E.,

Amemiya, A., Bean, L. J. H., Bird, T. D., Ledbetter, N., Mefford, H. C., Smith, R. J. H., and Stephens, K. eds.), Seattle (WA). pp

63. Plebani, A., Soresina, A., Rondelli, R., Amato, G. M., Azzari, C., Cardinale, F., Cazzola, G., Consolini, R., De Mattia, D., Dell'Erba, G., Duse, M., Fiorini, M., Martino, S., Martire, B., Masi, M., Monafo, V., Moschese, V., Notarangelo, L. D., Orlandi, P., Panei, P., Pession, A., Pietrogrande, M. C., Pignata, C., Quinti, I., Ragno, V., Rossi, P., Sciotto, A., Stabile, A., and Italian Pediatric Group for, X.-A. (2002) Clinical, immunological, and molecular analysis in a large cohort of patients with X-linked agammaglobulinemia: an Italian multicenter study. *Clin Immunol* **104**, 221-230

64. Rawlings, D. J., Saffran, D. C., Tsukada, S., Largaespada, D. A., Grimaldi, J. C., Cohen, L., Mohr, R. N., Bazan, J. F., Howard, M., Copeland, N. G., and et al. (1993) Mutation of unique region of Bruton's tyrosine kinase in immunodeficient XID mice. *Science* **261**, 358-361

65. Thomas, J. D., Sideras, P., Smith, C. I., Vorechovsky, I., Chapman, V., and Paul, W. E. (1993) Colocalization of X-linked agammaglobulinemia and X-linked immunodeficiency genes. *Science* **261**, 355-358

66. Li, T., Rawlings, D. J., Park, H., Kato, R. M., Witte, O. N., and Satterthwaite, A. B. (1997) Constitutive membrane association potentiates activation of Bruton tyrosine kinase. *Oncogene* **15**, 1375-1383

67. Treanor, B. (2012) B-cell receptor: from resting state to activate. *Immunology* **136**, 21-27

68. Reth, M. (1995) The B-cell antigen receptor complex and co-receptors. *Immunol Today* **16**, 310-313

69. Rawlings, D. J., Scharenberg, A. M., Park, H., Wahl, M. I., Lin, S., Kato, R. M., Fluckiger, A. C., Witte, O. N., and Kinet, J. P. (1996) Activation of BTK by a phosphorylation mechanism initiated by SRC family kinases. *Science* **271**, 822-825

70. Varnai, P., Rother, K. I., and Balla, T. (1999) Phosphatidylinositol 3-kinase-dependent membrane association of the Bruton's tyrosine kinase pleckstrin homology domain visualized in single living cells. *J Biol Chem* **274**, 10983-10989

71. Campbell, K. S. (1999) Signal transduction from the B cell antigen-receptor. *Curr Opin Immunol* **11**, 256-264

72. Humphries, L. A., Dangelmaier, C., Sommer, K., Kipp, K., Kato, R. M., Griffith, N., Bakman, I., Turk, C. W., Daniel, J. L., and Rawlings, D. J. (2004) Tec kinases mediate sustained calcium influx via site-specific tyrosine phosphorylation of the phospholipase Cgamma Src homology 2-Src homology 3 linker. *J Biol Chem* **279**, 37651-37661

73. Fluckiger, A. C., Li, Z., Kato, R. M., Wahl, M. I., Ochs, H. D., Longnecker, R., Kinet, J. P., Witte, O. N., Scharenberg, A. M., and Rawlings, D. J. (1998) Btk/Tec kinases regulate sustained increases in intracellular Ca2+ following B-cell receptor activation. *EMBO J* **17**, 1973-1985

74. Niemann, C. U., and Wiestner, A. (2013) B-cell receptor signaling as a driver of lymphoma development and evolution. *Semin Cancer Biol* **23**, 410-421

75. Young, R. M., and Staudt, L. M. (2013) Targeting pathological B cell receptor signalling in lymphoid malignancies. *Nat Rev Drug Discov* **12**, 229-243

76. Buggy, J. J., and Elias, L. (2012) Bruton tyrosine kinase (BTK) and its role in B-cell malignancy. *Int Rev Immunol* **31**, 119-132

77. Woyach, J. A., Bojnik, E., Ruppert, A. S., Stefanovski, M. R., Goettl, V. M., Smucker, K. A., Smith, L. L., Dubovsky, J. A., Towns, W. H., MacMurray, J., Harrington, B. K., Davis, M. E., Gobessi, S., Laurenti, L., Chang, B. Y., Buggy, J. J., Efremov, D. G., Byrd, J. C., and Johnson, A. J. (2014) Bruton's tyrosine kinase (BTK) function is important to the development and expansion of chronic lymphocytic leukemia (CLL). *Blood* **123**, 1207-1213

78. de Gorter, D. J., Beuling, E. A., Kersseboom, R., Middendorp, S., van Gils, J. M., Hendriks, R. W., Pals, S. T., and Spaargaren, M. (2007) Bruton's tyrosine kinase and phospholipase Cgamma2 mediate chemokine-controlled B cell migration and homing. *Immunity* **26**, 93-104

79. Ortolano, S., Hwang, I. Y., Han, S. B., and Kehrl, J. H. (2006) Roles for phosphoinositide 3-kinases, Bruton's tyrosine kinase, and Jun kinases in B lymphocyte chemotaxis and homing. *Eur J Immunol* **36**, 1285-1295

80. Quiroga, M. P., Balakrishnan, K., Kurtova, A. V., Sivina, M., Keating, M. J., Wierda, W. G., Gandhi, V., and Burger, J. A. (2009) B-cell antigen receptor signaling enhances chronic lymphocytic leukemia cell migration and survival: specific targeting with a novel spleen tyrosine kinase inhibitor, R406. *Blood* **114**, 1029-1037

81. Ponader, S., Chen, S. S., Buggy, J. J., Balakrishnan, K., Gandhi, V., Wierda, W. G., Keating, M. J., O'Brien, S., Chiorazzi, N., and Burger, J. A. (2012) The Bruton tyrosine kinase inhibitor PCI-32765 thwarts chronic lymphocytic leukemia cell survival and tissue homing in vitro and in vivo. *Blood* **119**, 1182-1189

82. Burger, J. A., Ghia, P., Rosenwald, A., and Caligaris-Cappio, F. (2009) The microenvironment in mature B-cell malignancies: a target for new treatment strategies. *Blood* **114**, 3367-3375

83. Herman, S. E., Gordon, A. L., Hertlein, E., Ramanunni, A., Zhang, X., Jaglowski, S., Flynn, J., Jones, J., Blum, K. A., Buggy, J. J., Hamdy, A., Johnson, A. J., and Byrd, J. C. (2011) Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood* **117**, 6287-6296

84. Pan, Z., Scheerens, H., Li, S. J., Schultz, B. E., Sprengeler, P. A., Burrill, L. C., Mendonca, R. V., Sweeney, M. D., Scott, K. C., Grothaus, P. G., Jeffery, D. A., Spoerke, J. M., Honigberg, L. A., Young, P. R., Dalrymple, S. A., and Palmer, J. T. (2007) Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. *ChemMedChem* **2**, 58-61

85. Honigberg, L. A., Smith, A. M., Sirisawad, M., Verner, E., Loury, D., Chang, B., Li, S., Pan, Z., Thamm, D. H., Miller, R. A., and Buggy, J. J. (2010) The

Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. *Proc Natl Acad Sci U S A* **107**, 13075-13080

86. Novero, A., Ravella, P. M., Chen, Y., Dous, G., and Liu, D. (2014) Ibrutinib for B cell malignancies. *Exp Hematol Oncol* **3**, 4

87. Cameron, F., and Sanford, M. (2014) Ibrutinib: first global approval. *Drugs* **74**, 263-271

88. Burger, J. A., and Buggy, J. J. (2013) Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765). *Leuk Lymphoma* **54**, 2385-2391

89. Vargas, L., Hamasy, A., Nore, B. F., and Smith, C. I. (2013) Inhibitors of BTK and ITK: state of the new drugs for cancer, autoimmunity and inflammatory diseases. *Scand J Immunol* **78**, 130-139

90. Herman, S. E., Mustafa, R. Z., Gyamfi, J. A., Pittaluga, S., Chang, S., Chang, B., Farooqui, M., and Wiestner, A. (2014) Ibrutinib inhibits BCR and NF-kappaB signaling and reduces tumor proliferation in tissue-resident cells of patients with CLL. *Blood* **123**, 3286-3295

91. Singh, J., Petter, R. C., and Kluge, A. F. (2010) Targeted covalent drugs of the kinase family. *Curr Opin Chem Biol* **14**, 475-480

92. Advani, R. H., Buggy, J. J., Sharman, J. P., Smith, S. M., Boyd, T. E., Grant, B., Kolibaba, K. S., Furman, R. R., Rodriguez, S., Chang, B. Y., Sukbuntherng, J., Izumi, R., Hamdy, A., Hedrick, E., and Fowler, N. H. (2013) Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *J Clin Oncol* **31**, 88-94

93. Wang, M. L., Rule, S., Martin, P., Goy, A., Auer, R., Kahl, B. S., Jurczak, W., Advani, R. H., Romaguera, J. E., Williams, M. E., Barrientos, J. C., Chmielowska, E., Radford, J., Stilgenbauer, S., Dreyling, M., Jedrzejczak, W. W., Johnson, P., Spurgeon, S. E., Li, L., Zhang, L., Newberry, K., Ou, Z., Cheng, N., Fang, B., McGreivy, J., Clow, F., Buggy, J. J., Chang, B. Y., Beaupre, D. M., Kunkel, L. A., and Blum, K. A. (2013) Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *N Engl J Med* **369**, 507-516

94. Treon, S. P., Tripsas, C. K., Meid, K., Warren, D., Varma, G., Green, R., Argyropoulos, K. V., Yang, G., Cao, Y., Xu, L., Patterson, C. J., Rodig, S., Zehnder, J. L., Aster, J. C., Harris, N. L., Kanan, S., Ghobrial, I., Castillo, J. J., Laubach, J. P., Hunter, Z. R., Salman, Z., Li, J., Cheng, M., Clow, F., Graef, T., Palomba, M. L., and Advani, R. H. (2015) Ibrutinib in previously treated Waldenstrom's macroglobulinemia. *N Engl J Med* **372**, 1430-1440

95. Kim, E. S., and Dhillon, S. (2015) Ibrutinib: a review of its use in patients with mantle cell lymphoma or chronic lymphocytic leukaemia. *Drugs* **75**, 769-776

96. Chang, B. Y., Huang, M. M., Francesco, M., Chen, J., Sokolove, J., Magadala, P., Robinson, W. H., and Buggy, J. J. (2011) The Bruton tyrosine kinase inhibitor PCI-32765 ameliorates autoimmune arthritis by inhibition of multiple effector cells. *Arthritis Res Ther* **13**, R115

97. Marostica, E., Sukbuntherng, J., Loury, D., de Jong, J., de Trixhe, X. W., Vermeulen, A., De Nicolao, G., O'Brien, S., Byrd, J. C., Advani, R., McGreivy, J., and Poggesi, I. (2015) Population pharmacokinetic model of ibrutinib, a Bruton tyrosine kinase inhibitor, in patients with B cell malignancies. *Cancer Chemother Pharmacol* **75**, 111-121

98. Burger, J. A. (2014) Bruton's tyrosine kinase (BTK) inhibitors in clinical trials. *Curr Hematol Malig Rep* **9**, 44-49

99. Byrd, J. C., Furman, R. R., Coutre, S. E., Burger, J. A., Blum, K. A., Coleman, M., Wierda, W. G., Jones, J. A., Zhao, W., Heerema, N. A., Johnson, A. J., Shaw, Y., Bilotti, E., Zhou, C., James, D. F., and O'Brien, S. (2015) Three-year follow-up of treatment-naive and previously treated patients with CLL and SLL receiving single-agent ibrutinib. *Blood* **125**, 2497-2506

100. Brown, J. R. (2013) Ibrutinib (PCI-32765), the first BTK (Bruton's tyrosine kinase) inhibitor in clinical trials. *Curr Hematol Malig Rep* **8**, 1-6

101. Berglof, A., Hamasy, A., Meinke, S., Palma, M., Krstic, A., Mansson, R., Kimby, E., Osterborg, A., and Smith, C. I. (2015) Targets for Ibrutinib Beyond B Cell Malignancies. *Scand J Immunol* **82**, 208-217

102. Zhang, S. Q., Smith, S. M., Zhang, S. Y., and Lynn Wang, Y. (2015) Mechanisms of ibrutinib resistance in chronic lymphocytic leukaemia and non-Hodgkin lymphoma. *Br J Haematol* **170**, 445-456

103. Woyach, J. A., and Johnson, A. J. (2015) Targeted therapies in CLL: mechanisms of resistance and strategies for management. *Blood* **126**, 471-477

104. Chiron, D., Di Liberto, M., Martin, P., Huang, X., Sharman, J., Blecua, P., Mathew, S., Vijay, P., Eng, K., Ali, S., Johnson, A., Chang, B., Ely, S., Elemento, O., Mason, C. E., Leonard, J. P., and Chen-Kiang, S. (2014) Cell-cycle reprogramming for PI3K inhibition overrides a relapse-specific C481S BTK mutation revealed by longitudinal functional genomics in mantle cell lymphoma. *Cancer Discov* **4**, 1022-1035

105. Cheah, C. Y., Chihara, D., Romaguera, J. E., Fowler, N. H., Seymour, J. F., Hagemeister, F. B., Champlin, R. E., and Wang, M. L. (2015) Patients with mantle cell lymphoma failing ibrutinib are unlikely to respond to salvage chemotherapy and have poor outcomes. *Ann Oncol* **26**, 1175-1179

106. Furman, R. R., Cheng, S., Lu, P., Setty, M., Perez, A. R., Guo, A., Racchumi, J., Xu, G., Wu, H., Ma, J., Steggerda, S. M., Coleman, M., Leslie, C., and Wang, Y. L. (2014) Ibrutinib resistance in chronic lymphocytic leukemia. *N Engl J Med* **370**, 2352-2354

107. Maddocks, K. J., Ruppert, A. S., Lozanski, G., Heerema, N. A., Zhao, W., Abruzzo, L., Lozanski, A., Davis, M., Gordon, A., Smith, L. L., Mantel, R., Jones, J. A., Flynn, J. M., Jaglowski, S. M., Andritsos, L. A., Awan, F., Blum, K. A., Grever, M. R., Johnson, A. J., Byrd, J. C., and Woyach, J. A. (2015) Etiology of Ibrutinib Therapy Discontinuation and Outcomes in Patients With Chronic Lymphocytic Leukemia. *JAMA Oncol* **1**, 80-87

108. Liu, T. M., Woyach, J. A., Zhong, Y., Lozanski, A., Lozanski, G., Dong, S., Strattan, E., Lehman, A., Zhang, X., Jones, J. A., Flynn, J., Andritsos, L. A.,

Maddocks, K., Jaglowski, S. M., Blum, K. A., Byrd, J. C., Dubovsky, J. A., and Johnson, A. J. (2015) Hypermorphic mutation of phospholipase C, gamma2 acquired in ibrutinib-resistant CLL confers BTK independency upon B-cell receptor activation. *Blood* **126**, 61-68

109. Albitar, A., Ma, W., DeDios, I., Estella, J., Ahn, I., Farooqui, M., Wiestner, A., and Albitar, M. (2017) Using high-sensitivity sequencing for the detection of mutations in BTK and PLCgamma2 genes in cellular and cell-free DNA and correlation with progression in patients treated with BTK inhibitors. *Oncotarget*

110. Sharma, S., Galanina, N., Guo, A., Lee, J., Kadri, S., Van Slambrouck, C., Long, B., Wang, W., Ming, M., Furtado, L. V., Segal, J. P., Stock, W., Venkataraman, G., Tang, W. J., Lu, P., and Wang, Y. L. (2016) Identification of a structurally novel BTK mutation that drives ibrutinib resistance in CLL. *Oncotarget* **7**, 68833-68841

111. Cao, Y., Hunter, Z. R., Liu, X., Xu, L., Yang, G., Chen, J., Patterson, C. J., Tsakmaklis, N., Kanan, S., Rodig, S., Castillo, J. J., and Treon, S. P. (2015) The WHIM-like CXCR4(S338X) somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenstrom's Macroglobulinemia. *Leukemia* **29**, 169-176

112. Wilson, W. H., Young, R. M., Schmitz, R., Yang, Y., Pittaluga, S., Wright, G., Lih, C. J., Williams, P. M., Shaffer, A. L., Gerecitano, J., de Vos, S., Goy, A., Kenkre, V. P., Barr, P. M., Blum, K. A., Shustov, A., Advani, R., Fowler, N. H., Vose, J. M., Elstrom, R. L., Habermann, T. M., Barrientos, J. C., McGreivy, J., Fardis, M., Chang, B. Y., Clow, F., Munneke, B., Moussa, D., Beaupre, D. M., and Staudt, L. M. (2015) Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat Med* **21**, 922-926

113. Ailawadhi, S., Paulus, A., and Chanan-Khan, A. (2016) Preclinical models of Waldenstrom's macroglobulinemia and drug resistance. *Best Pract Res Clin Haematol* **29**, 169-178

114. Wu, J., Liu, C., Tsui, S. T., and Liu, D. (2016) Second-generation inhibitors of Bruton tyrosine kinase. *J Hematol Oncol* **9**, 80

115. Byrd, J. C., Harrington, B., O'Brien, S., Jones, J. A., Schuh, A., Devereux, S., Chaves, J., Wierda, W. G., Awan, F. T., Brown, J. R., Hillmen, P., Stephens, D. M., Ghia, P., Barrientos, J. C., Pagel, J. M., Woyach, J., Johnson, D., Huang, J., Wang, X., Kaptein, A., Lannutti, B. J., Covey, T., Fardis, M., McGreivy, J., Hamdy, A., Rothbaum, W., Izumi, R., Diacovo, T. G., Johnson, A. J., and Furman, R. R. (2016) Acalabrutinib (ACP-196) in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med* **374**, 323-332

116. Wu, J., Zhang, M., and Liu, D. (2016) Acalabrutinib (ACP-196): a selective second-generation BTK inhibitor. *J Hematol Oncol* **9**, 21

117. Patel, V., Balakrishnan, K., Bibikova, E., Ayres, M., Keating, M. J., Wierda, W., and Gandhi, V. (2016) Comparison of acalabrutinib, a selective Bruton tyrosine kinase inhibitor, with ibrutinib in chronic lymphocytic leukemia cells. *Clin Cancer Res*

118. Johnson, A. R., Kohli, P. B., Katewa, A., Gogol, E., Belmont, L. D., Choy, R., Penuel, E., Burton, L., Eigenbrot, C., Yu, C., Ortwine, D. F., Bowman, K., Franke, Y., Tam, C., Estevez, A., Mortara, K., Wu, J., Li, H., Lin, M., Bergeron, P., Crawford, J. J., and Young, W. B. (2016) Battling Btk Mutants With Noncovalent Inhibitors That Overcome Cys481 and Thr474 Mutations. *ACS Chem Biol* **11**, 2897-2907

119. Berg, L. J., Finkelstein, L. D., Lucas, J. A., and Schwartzberg, P. L. (2005) Tec family kinases in T lymphocyte development and function. *Annu Rev Immunol* **23**, 549-600

120. Readinger, J. A., Mueller, K. L., Venegas, A. M., Horai, R., and Schwartzberg, P. L. (2009) Tec kinases regulate T-lymphocyte development and function: new insights into the roles of Itk and Rlk/Txk. *Immunol Rev* **228**, 93-114

121. Qi, Q., Kannan, A. K., and August, A. (2011) Tec family kinases: Itk signaling and the development of NKT alphabeta and gammadelta T cells. *FEBS J* **278**, 1970-1979

122. Prince, A. L., Yin, C. C., Enos, M. E., Felices, M., and Berg, L. J. (2009) The Tec kinases Itk and Rlk regulate conventional versus innate T-cell development. *Immunol Rev* **228**, 115-131

123. Gomez-Rodriguez, J., Kraus, Z. J., and Schwartzberg, P. L. (2011) Tec family kinases Itk and Rlk / Txk in T lymphocytes: cross-regulation of cytokine production and T-cell fates. *FEBS J* **278**, 1980-1989

124. Huck, K., Feyen, O., Niehues, T., Ruschendorf, F., Hubner, N., Laws, H. J., Telieps, T., Knapp, S., Wacker, H. H., Meindl, A., Jumaa, H., and Borkhardt, A. (2009) Girls homozygous for an IL-2-inducible T cell kinase mutation that leads to protein deficiency develop fatal EBV-associated lymphoproliferation. *J Clin Invest* **119**, 1350-1358

125. Czar, M. J., Debnath, J., Schaeffer, E. M., Lewis, C. M., and Schwartzberg, P. L. (2001) Biochemical and genetic analyses of the Tec kinases Itk and Rlk/Txk. *Biochem Soc Trans* **29**, 863-867

126. Andreotti, A. H., Schwartzberg, P. L., Joseph, R. E., and Berg, L. J. (2010) T-cell signaling regulated by the Tec family kinase, Itk. *Cold Spring Harb Perspect Biol* **2**, a002287

127. Tsoukas, C. D., Grasis, J. A., Ching, K. A., Kawakami, Y., and Kawakami, T. (2001) Itk/Emt: a link between T cell antigen receptor-mediated Ca2+ events and cytoskeletal reorganization. *Trends Immunol* **22**, 17-20

128. Sahu, N., and August, A. (2009) ITK inhibitors in inflammation and immune-mediated disorders. *Curr Top Med Chem* **9**, 690-703

129. Norman, P. (2014) Inducible tyrosine kinase inhibitors: a review of the patent literature (2010 - 2013). *Expert Opin Ther Pat* **24**, 979-991

130. Cho, H. S., Shin, H. M., Haberstock-Debic, H., Xing, Y., Owens, T. D., Funk, J. O., Hill, R. J., Bradshaw, J. M., and Berg, L. J. (2015) A Small Molecule Inhibitor of ITK and RLK Impairs Th1 Differentiation and Prevents Colitis Disease Progression. *J Immunol* **195**, 4822-4831

131. Mocsai, A., Ruland, J., and Tybulewicz, V. L. (2010) The SYK tyrosine kinase: a crucial player in diverse biological functions. *Nat Rev Immunol* **10**, 387-402

132. Riccaboni, M., Bianchi, I., and Petrillo, P. (2010) Spleen tyrosine kinases: biology, therapeutic targets and drugs. *Drug Discov Today* **15**, 517-530

133. Singh, R., Masuda, E. S., and Payan, D. G. (2012) Discovery and development of spleen tyrosine kinase (SYK) inhibitors. *J Med Chem* **55**, 3614-3643

134. Turner, M., Mee, P. J., Costello, P. S., Williams, O., Price, A. A., Duddy, L. P., Furlong, M. T., Geahlen, R. L., and Tybulewicz, V. L. (1995) Perinatal lethality and blocked B-cell development in mice lacking the tyrosine kinase Syk. *Nature* **378**, 298-302

135. Sada, K., Takano, T., Yanagi, S., and Yamamura, H. (2001) Structure and function of Syk protein-tyrosine kinase. *J Biochem* **130**, 177-186

136. Arias-Palomo, E., Recuero-Checa, M. A., Bustelo, X. R., and Llorca, O. (2007) 3D structure of Syk kinase determined by single-particle electron microscopy. *Biochim Biophys Acta* **1774**, 1493-1499

137. Lowell, C. A. (2011) Src-family and Syk kinases in activating and inhibitory pathways in innate immune cells: signaling cross talk. *Cold Spring Harb Perspect Biol* **3**

138. Bradshaw, J. M. (2010) The Src, Syk, and Tec family kinases: distinct types of molecular switches. *Cell Signal* **22**, 1175-1184

139. Gross, O., Poeck, H., Bscheider, M., Dostert, C., Hannesschlager, N., Endres, S., Hartmann, G., Tardivel, A., Schweighoffer, E., Tybulewicz, V., Mocsai, A., Tschopp, J., and Ruland, J. (2009) Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. *Nature* **459**, 433-436

140. Underhill, D. M., Rossnagle, E., Lowell, C. A., and Simmons, R. M. (2005) Dectin-1 activates Syk tyrosine kinase in a dynamic subset of macrophages for reactive oxygen production. *Blood* **106**, 2543-2550

141. Spalton, J. C., Mori, J., Pollitt, A. Y., Hughes, C. E., Eble, J. A., and Watson, S. P. (2009) The novel Syk inhibitor R406 reveals mechanistic differences in the initiation of GPVI and CLEC-2 signaling in platelets. *J Thromb Haemost* **7**, 1192-1199

142. Arias-Palomo, E., Recuero-Checa, M. A., Bustelo, X. R., and Llorca, O. (2009) Conformational rearrangements upon Syk auto-phosphorylation. *Biochim Biophys Acta* **1794**, 1211-1217

143. Tsang, E., Giannetti, A. M., Shaw, D., Dinh, M., Tse, J. K., Gandhi, S., Ho, H., Wang, S., Papp, E., and Bradshaw, J. M. (2008) Molecular mechanism of the Syk activation switch. *J Biol Chem* **283**, 32650-32659

144. Carsetti, L., Laurenti, L., Gobessi, S., Longo, P. G., Leone, G., and Efremov, D. G. (2009) Phosphorylation of the activation loop tyrosines is required for sustained Syk signaling and growth factor-independent B-cell proliferation. *Cell Signal* **21**, 1187-1194

145. Stepanek, O., Draber, P., Drobek, A., Horejsi, V., and Brdicka, T. (2013) Nonredundant roles of Src-family kinases and Syk in the initiation of B-cell antigen receptor signaling. *J Immunol* **190**, 1807-1818

146. Moon, K. D., Post, C. B., Durden, D. L., Zhou, Q., De, P., Harrison, M. L., and Geahlen, R. L. (2005) Molecular basis for a direct interaction between the Syk protein-tyrosine kinase and phosphoinositide 3-kinase. *J Biol Chem* **280**, 1543-1551

147. Geahlen, R. L. (2009) Syk and pTyr'd: Signaling through the B cell antigen receptor. *Biochim Biophys Acta* **1793**, 1115-1127

148. Katkere, B., Rosa, S., and Drake, J. R. (2012) The Syk-binding ubiquitin ligase c-Cbl mediates signaling-dependent B cell receptor ubiquitination and B cell receptor-mediated antigen processing and presentation. *J Biol Chem* **287**, 16636-16644

149. Keshvara, L. M., Isaacson, C. C., Yankee, T. M., Sarac, R., Harrison, M. L., and Geahlen, R. L. (1998) Syk- and Lyn-dependent phosphorylation of Syk on multiple tyrosines following B cell activation includes a site that negatively regulates signaling. *J Immunol* **161**, 5276-5283

150. Juszczynski, P., Chen, L., O'Donnell, E., Polo, J. M., Ranuncolo, S. M., Dalla-Favera, R., Melnick, A., and Shipp, M. A. (2009) BCL6 modulates tonic BCR signaling in diffuse large B-cell lymphomas by repressing the SYK phosphatase, PTPROt. *Blood* **114**, 5315-5321

151. Latour, S., Zhang, J., Siraganian, R. P., and Veillette, A. (1998) A unique insert in the linker domain of Syk is necessary for its function in immunoreceptor signalling. *EMBO J* **17**, 2584-2595

152. Zhou, F., Hu, J., Ma, H., Harrison, M. L., and Geahlen, R. L. (2006) Nucleocytoplasmic trafficking of the Syk protein tyrosine kinase. *Mol Cell Biol* **26**, 3478-3491

153. Hong, J., Hu, K., Yuan, Y., Sang, Y., Bu, Q., Chen, G., Yang, L., Li, B., Huang, P., Chen, D., Liang, Y., Zhang, R., Pan, J., Zeng, Y. X., and Kang, T. (2012) CHK1 targets spleen tyrosine kinase (L) for proteolysis in hepatocellular carcinoma. *J Clin Invest* **122**, 2165-2175

154. Buchner, M., Fuchs, S., Prinz, G., Pfeifer, D., Bartholome, K., Burger, M., Chevalier, N., Vallat, L., Timmer, J., Gribben, J. G., Jumaa, H., Veelken, H., Dierks, C., and Zirlik, K. (2009) Spleen tyrosine kinase is overexpressed and represents a potential therapeutic target in chronic lymphocytic leukemia. *Cancer Res* **69**, 5424-5432

155. Feldman, A. L., Sun, D. X., Law, M. E., Novak, A. J., Attygalle, A. D., Thorland, E. C., Fink, S. R., Vrana, J. A., Caron, B. L., Morice, W. G., Remstein, E. D., Grogg, K. L., Kurtin, P. J., Macon, W. R., and Dogan, A. (2008) Overexpression of Syk tyrosine kinase in peripheral T-cell lymphomas. *Leukemia* **22**, 1139-1143

156. Young, R. M., Hardy, I. R., Clarke, R. L., Lundy, N., Pine, P., Turner, B. C., Potter, T. A., and Refaeli, Y. (2009) Mouse models of non-Hodgkin lymphoma reveal Syk as an important therapeutic target. *Blood* **113**, 2508-2516

157. Chen, L., Monti, S., Juszczynski, P., Daley, J., Chen, W., Witzig, T. E., Habermann, T. M., Kutok, J. L., and Shipp, M. A. (2008) SYK-dependent tonic B-cell receptor signaling is a rational treatment target in diffuse large B-cell lymphoma. *Blood* **111**, 2230-2237

158. Kuno, Y., Abe, A., Emi, N., Iida, M., Yokozawa, T., Towatari, M., Tanimoto, M., and Saito, H. (2001) Constitutive kinase activation of the TEL-Syk fusion gene in myelodysplastic syndrome with t(9;12)(q22;p12). *Blood* **97**, 1050-1055

159. Sung, Y. M., Xu, X., Sun, J., Mueller, D., Sentissi, K., Johnson, P., Urbach, E., Seillier-Moiseiwitsch, F., Johnson, M. D., and Mueller, S. C. (2009) Tumor suppressor function of Syk in human MCF10A in vitro and normal mouse mammary epithelium in vivo. *PLoS One* **4**, e7445

160. Tan, S. L., Liao, C., Lucas, M. C., Stevenson, C., and DeMartino, J. A. (2013) Targeting the SYK-BTK axis for the treatment of immunological and hematological disorders: recent progress and therapeutic perspectives. *Pharmacol Ther* **138**, 294-309

161. Friedberg, J. W., Sharman, J., Sweetenham, J., Johnston, P. B., Vose, J. M., Lacasce, A., Schaefer-Cutillo, J., De Vos, S., Sinha, R., Leonard, J. P., Cripe, L. D., Gregory, S. A., Sterba, M. P., Lowe, A. M., Levy, R., and Shipp, M. A. (2010) Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. *Blood* **115**, 2578-2585

162. Buchner, M., Baer, C., Prinz, G., Dierks, C., Burger, M., Zenz, T., Stilgenbauer, S., Jumaa, H., Veelken, H., and Zirlik, K. (2010) Spleen tyrosine kinase inhibition prevents chemokine- and integrin-mediated stromal protective effects in chronic lymphocytic leukemia. *Blood* **115**, 4497-4506

163. Huang, Y., Moreau, A., Dupuis, J., Streubel, B., Petit, B., Le Gouill, S., Martin-Garcia, N., Copie-Bergman, C., Gaillard, F., Qubaja, M., Fabiani, B., Roncador, G., Haioun, C., Delfau-Larue, M. H., Marafioti, T., Chott, A., and Gaulard, P. (2009) Peripheral T-cell lymphomas with a follicular growth pattern are derived from follicular helper T cells (TFH) and may show overlapping features with angioimmunoblastic T-cell lymphomas. *Am J Surg Pathol* **33**, 682-690

164. Hussain, A., Faryal, R., Nore, B. F., Mohamed, A. J., and Smith, C. I. (2009) Phosphatidylinositol-3-kinase-dependent phosphorylation of SLP-76 by the lymphoma-associated ITK-SYK fusion-protein. *Biochem Biophys Res Commun* **390**, 892-896

165. Hussain, A., Yu, L., Faryal, R., Mohammad, D. K., Mohamed, A. J., and Smith, C. I. (2011) TEC family kinases in health and disease--loss-of-function of BTK and ITK and the gain-of-function fusions ITK-SYK and BTK-SYK. *FEBS J* **278**, 2001-2010

166. Mulloy, J. C. (2010) Peripheral T cell lymphoma: new model + new insight. *J Exp Med* **207**, 911-913

167. Rigby, S., Huang, Y., Streubel, B., Chott, A., Du, M. Q., Turner, S. D., and Bacon, C. M. (2009) The lymphoma-associated fusion tyrosine kinase ITK-

SYK requires pleckstrin homology domain-mediated membrane localization for activation and cellular transformation. *J Biol Chem* **284**, 26871-26881

168. Bisig, B., Gaulard, P., and de Leval, L. (2012) New biomarkers in T-cell lymphomas. *Best Pract Res Clin Haematol* **25**, 13-28

169. Pechloff, K., Holch, J., Ferch, U., Schweneker, M., Brunner, K., Kremer, M., Sparwasser, T., Quintanilla-Martinez, L., Zimber-Strobl, U., Streubel, B., Gewies, A., Peschel, C., and Ruland, J. (2010) The fusion kinase ITK-SYK mimics a T cell receptor signal and drives oncogenesis in conditional mouse models of peripheral T cell lymphoma. *J Exp Med* **207**, 1031-1044

170. Bach, M. P., Hug, E., Werner, M., Holch, J., Sprissler, C., Pechloff, K., Zirlik, K., Zeiser, R., Dierks, C., Ruland, J., and Jumaa, H. (2014) Premature terminal differentiation protects from deregulated lymphocyte activation by ITK-Syk. *J Immunol* **192**, 1024-1033

171. Dierks, C., Adrian, F., Fisch, P., Ma, H., Maurer, H., Herchenbach, D., Forster, C. U., Sprissler, C., Liu, G., Rottmann, S., Guo, G. R., Katja, Z., Veelken, H., and Warmuth, M. (2010) The ITK-SYK fusion oncogene induces a T-cell lymphoproliferative disease in mice mimicking human disease. *Cancer Res* **70**, 6193-6204

172. Hussain, A., Mohammad, D. K., Gustafsson, M. O., Uslu, M., Hamasy, A., Nore, B. F., Mohamed, A. J., and Smith, C. I. (2013) Signaling of the ITK (interleukin 2-inducible T cell kinase)-SYK (spleen tyrosine kinase) fusion kinase is dependent on adapter SLP-76 and on the adapter function of the kinases SYK and ZAP70. *J Biol Chem* **288**, 7338-7350

173. De Braekeleer, E., Douet-Guilbert, N., Morel, F., Le Bris, M. J., Basinko, A., and De Braekeleer, M. (2012) ETV6 fusion genes in hematological malignancies: a review. *Leuk Res* **36**, 945-961

174. Eguchi, M., Eguchi-Ishimae, M., Tojo, A., Morishita, K., Suzuki, K., Sato, Y., Kudoh, S., Tanaka, K., Setoyama, M., Nagamura, F., Asano, S., and Kamada, N. (1999) Fusion of ETV6 to neurotrophin-3 receptor TRKC in acute myeloid leukemia with t(12;15)(p13;q25). *Blood* **93**, 1355-1363

175. Lacronique, V., Boureux, A., Valle, V. D., Poirel, H., Quang, C. T., Mauchauffe, M., Berthou, C., Lessard, M., Berger, R., Ghysdael, J., and Bernard, O. A. (1997) A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. *Science* **278**, 1309-1312

176. Papadopoulos, P., Ridge, S. A., Boucher, C. A., Stocking, C., and Wiedemann, L. M. (1995) The novel activation of ABL by fusion to an ets-related gene, TEL. *Cancer Res* **55**, 34-38

177. Yagasaki, F., Wakao, D., Yokoyama, Y., Uchida, Y., Murohashi, I., Kayano, H., Taniwaki, M., Matsuda, A., and Bessho, M. (2001) Fusion of ETV6 to fibroblast growth factor receptor 3 in peripheral T-cell lymphoma with a t(4;12)(p16;p13) chromosomal translocation. *Cancer Res* **61**, 8371-8374

178. Sprissler, C., Belenki, D., Maurer, H., Aumann, K., Pfeifer, D., Klein, C., Muller, T. A., Kissel, S., Hulsdunker, J., Alexandrovski, J., Brummer, T., Jumaa, H., Duyster, J., and Dierks, C. (2014) Depletion of STAT5 blocks TEL-

SYK-induced APMF-type leukemia with myelofibrosis and myelodysplasia in mice. *Blood Cancer J* **4**, e240

179. Kanie, T., Abe, A., Matsuda, T., Kuno, Y., Towatari, M., Yamamoto, T., Saito, H., Emi, N., and Naoe, T. (2004) TEL-Syk fusion constitutively activates PI3-K/Akt, MAPK and JAK2-independent STAT5 signal pathways. *Leukemia* **18**, 548-555

180. Graham, M. T., Abram, C. L., Hu, Y., and Lowell, C. A. (2013) Expression of the TEL-Syk fusion protein in hematopoietic stem cells leads to rapidly fatal myelofibrosis in mice. *PLoS One* **8**, e77542

181. Notarangelo, L. D. (2010) Primary immunodeficiencies. *J Allergy Clin Immunol* **125**, S182-194

182. Monticelli, M., Ferro, T., Jaeken, J., Dos Reis Ferreira, V., and Videira, P. A. (2016) Immunological aspects of congenital disorders of glycosylation (CDG): a review. *J Inherit Metab Dis* **39**, 765-780

183. Marodi, L., and Notarangelo, L. D. (2007) Immunological and genetic bases of new primary immunodeficiencies. *Nat Rev Immunol* **7**, 851-861

184. Bonilla, F. A., Bernstein, I. L., Khan, D. A., Ballas, Z. K., Chinen, J., Frank, M. M., Kobrynski, L. J., Levinson, A. I., Mazer, B., Nelson, R. P., Jr., Orange, J. S., Routes, J. M., Shearer, W. T., Sorensen, R. U., American Academy of Allergy, A., Immunology, American College of Allergy, A., Immunology, Joint Council of Allergy, A., and Immunology. (2005) Practice parameter for the diagnosis and management of primary immunodeficiency. *Ann Allergy Asthma Immunol* **94**, S1-63

185. Notarangelo, L. D. (2010) PIDs and cancer: an evolving story. *Blood* **116**, 1189-1190

186. Moens, L. N., Falk-Sorqvist, E., Asplund, A. C., Bernatowska, E., Smith, C. I., and Nilsson, M. (2014) Diagnostics of primary immunodeficiency diseases: a sequencing capture approach. *PLoS One* **9**, e114901

187. Al-Herz, W., Bousfiha, A., Casanova, J. L., Chatila, T., Conley, M. E., Cunningham-Rundles, C., Etzioni, A., Franco, J. L., Gaspar, H. B., Holland, S. M., Klein, C., Nonoyama, S., Ochs, H. D., Oksenhendler, E., Picard, C., Puck, J. M., Sullivan, K., and Tang, M. L. (2014) Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. *Front Immunol* **5**, 162

188. Al-Herz, W., Aldhekri, H., Barbouche, M. R., and Rezaei, N. (2014) Consanguinity and primary immunodeficiencies. *Hum Hered* **77**, 138-143

189. Varki, A. (1993) Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* **3**, 97-130

190. Kornfeld, R., and Kornfeld, S. (1985) Assembly of asparagine-linked oligosaccharides. *Annu Rev Biochem* **54**, 631-664

191. Grunewald, S., Matthijs, G., and Jaeken, J. (2002) Congenital disorders of glycosylation: a review. *Pediatr Res* **52**, 618-624

192. Chinen, J., Notarangelo, L. D., and Shearer, W. T. (2015) Advances in basic and clinical immunology in 2014. *J Allergy Clin Immunol* **135**, 1132-1141

193. Greig, K. T., Antonchuk, J., Metcalf, D., Morgan, P. O., Krebs, D. L., Zhang, J. G., Hacking, D. F., Bode, L., Robb, L., Kranz, C., de Graaf, C., Bahlo, M., Nicola, N. A., Nutt, S. L., Freeze, H. H., Alexander, W. S., Hilton, D. J., and Kile, B. T. (2007) Agm1/Pgm3-mediated sugar nucleotide synthesis is essential for hematopoiesis and development. *Mol Cell Biol* **27**, 5849-5859

194. Pang, H., Li, Z., Wang, B., and Ding, M. (2010) Polymorphic analysis of the human phosphoglucomutase-3 gene based on mismatched PCR-RFLP technique. *Biochem Genet* **48**, 208-214

195. Wu, G., Hitchen, P. G., Panico, M., North, S. J., Barbouche, M. R., Binet, D., Morris, H. R., Dell, A., and Haslam, S. M. (2016) Glycoproteomic studies of IgE from a novel hyper IgE syndrome linked to PGM3 mutation. *Glycoconj J* **33**, 447-456

196. Stray-Pedersen, A., Backe, P. H., Sorte, H. S., Morkrid, L., Chokshi, N. Y., Erichsen, H. C., Gambin, T., Elgstoen, K. B., Bjoras, M., Wlodarski, M. W., Kruger, M., Jhangiani, S. N., Muzny, D. M., Patel, A., Raymond, K. M., Sasa, G. S., Krance, R. A., Martinez, C. A., Abraham, S. M., Speckmann, C., Ehl, S., Hall, P., Forbes, L. R., Merckoll, E., Westvik, J., Nishimura, G., Rustad, C. F., Abrahamsen, T. G., Ronnestad, A., Osnes, L. T., Egeland, T., Rodningen, O. K., Beck, C. R., Baylor-Johns Hopkins Center for Mendelian, G., Boerwinkle, E. A., Gibbs, R. A., Lupski, J. R., Orange, J. S., Lausch, E., and Hanson, I. C. (2014) PGM3 mutations cause a congenital disorder of glycosylation with severe immunodeficiency and skeletal dysplasia. *Am J Hum Genet* **95**, 96-107

197. Sassi, A., Lazaroski, S., Wu, G., Haslam, S. M., Fliegauf, M., Mellouli, F., Patiroglu, T., Unal, E., Ozdemir, M. A., Jouhadi, Z., Khadir, K., Ben-Khemis, L., Ben-Ali, M., Ben-Mustapha, I., Borchani, L., Pfeifer, D., Jakob, T., Khemiri, M., Asplund, A. C., Gustafsson, M. O., Lundin, K. E., Falk-Sorqvist, E., Moens, L. N., Gungor, H. E., Engelhardt, K. R., Dziadzio, M., Stauss, H., Fleckenstein, B., Meier, R., Prayitno, K., Maul-Pavicic, A., Schaffer, S., Rakhmanov, M., Henneke, P., Kraus, H., Eibel, H., Kolsch, U., Nadifi, S., Nilsson, M., Bejaoui, M., Schaffer, A. A., Smith, C. I., Dell, A., Barbouche, M. R., and Grimbacher, B. (2014) Hypomorphic homozygous mutations in phosphoglucomutase 3 (PGM3) impair immunity and increase serum IgE levels. *J Allergy Clin Immunol* **133**, 1410-1419, 1419 e1411-1413

198. Carlson, R. J., Bond, M. R., Hutchins, S., Brown, Y., Wolfe, L. A., Lam, C., Nelson, C., DiMaggio, T., Jones, N., Rosenzweig, S. D., Stone, K. D., Freeman, A. F., Holland, S. M., Hanover, J. A., Milner, J. D., and Lyons, J. J. (2017) Detection of phosphoglucomutase-3 deficiency by lectin-based flow cytometry. *J Allergy Clin Immunol*

199. Yang, L., Fliegauf, M., and Grimbacher, B. (2014) Hyper-IgE syndromes: reviewing PGM3 deficiency. *Curr Opin Pediatr* **26**, 697-703

200. Zhang, Y., Yu, X., Ichikawa, M., Lyons, J. J., Datta, S., Lamborn, I. T., Jing, H., Kim, E. S., Biancalana, M., Wolfe, L. A., DiMaggio, T., Matthews, H. F., Kranick, S. M., Stone, K. D., Holland, S. M., Reich, D. S., Hughes, J. D.,

Mehmet, H., McElwee, J., Freeman, A. F., Freeze, H. H., Su, H. C., and Milner, J. D. (2014) Autosomal recessive phosphoglucomutase 3 (PGM3) mutations link glycosylation defects to atopy, immune deficiency, autoimmunity, and neurocognitive impairment. *J Allergy Clin Immunol* **133**, 1400-1409, 1409 e1401-1405

201. Hamasy, A., Wang, Q., Blomberg, K. E., Mohammad, D. K., Yu, L., Vihinen, M., Berglof, A., and Smith, C. I. (2017) Substitution scanning identifies a novel, catalytically active ibrutinib-resistant BTK cysteine 481 to threonine (C481T) variant. *Leukemia* **31**, 177-185